September 18, 2001

Public Information and Records Integrity Branch
Information Resources and Services Division (7502C)
Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Attention: Ms. Christina Scheltema, Chemical Review Manager
Special Review and Reregistration Division

Subject: **Sodium Acifluorfen (Docket Control Number OPP-34241)**:
Phase 3 Evaluation of Preliminary Human Health and Ecological Risk Assessment

Dear Ms. Scheltema:

BASF Corporation is hereby submitting a Phase 3 response to the preliminary risk assessment documents for sodium acifluorfen which were made available for public comment on July 26, 2001. BASF has previously responded (July 21, 2000) to an earlier version of these documents, but numerous of our comments were not incorporated into the preliminary risk assessment recently published. We are therefore resubmitting those revisions, as well as presenting corrections and comments to more substantive issues in this document and in its attachments. Presented below are our comments on the various chapters of the EPA preliminary assessment that have been provided in the public docket.

## PRELIMINARY HUMAN HEALTH RISK ASSESSMENT

### Health Effects Division Chapter for the Reregistration Eligibility Decision Document

1. In the memorandum that precedes the table of contents for this chapter, it is noted that a subchronic mouse study and hepatic cell proliferation study have been submitted. It should also be noted that three Ames assays and information needed to upgrade a mouse lymphoma assay have also been submitted. The MRID numbers assigned to the Ames assays are MRID 45393902, 45323501 and 45393901. The information needed to upgrade the mouse lymphoma assay has not been assigned an MRID number.

2. Page 3. **Rat developmental toxicity study**: The Agency states that a developmental neurotoxicity study in the rat is required based on increased incidence of dilated lateral ventricles of the brain in the rat developmental toxicity study (MRID 00122743). The reviewer states that this effect indicates that

administration of the compound results in neurotoxicity. First, it should be noted that dilated ventricles were described in the EPA reviews and summaries of this study as only "slightly dilated." Second, the slightly dilated ventricles are not likely to be true adverse effects, but are more likely secondary to decreased fetal body weight. Fetal body weights were decreased at the mid and high doses by 9% and 19% when compared to controls. Decreases in body weight are often associated with delayed development. In this study, this effect was evidenced in the pups by delayed skeletal ossifications and slight dilation of the lateral ventricles of the brain. At the mid dose of 90 mg/kg/day, the dilation of brain ventricles was observed in 10 pups from 8 litters. The average pup weight from each of these litters was below the control average by 14%. The mean pup weight for those pups with dilated ventricles in all dose groups was 11.5% below the mean pup weight for those pups without dilated ventricles in all dose groups.

| **DOSE (MG/KG)** | **MEAN PUP WEIGHT – ALL PUPS** | **MEAN PUP WEIGHT W/O DILATED VENTRICLES** | **MEAN PUP WEIGHT WITH DILATED VENTRICLES** | **PERCENT DIFFERENCE COMPARED TO PUPS W/O DILATED VENTRICLES** |
|---|---|---|---|---|
| Control | 3.72 ± 0.23 | 3.72 ± 0.23 | 3.09 ± N/A | -16.9% |
| 20 | 3.77 ± 0.27 | 3.77 ± 0.27 | 3.36 ± N/A | -10.9% |
| 90 | 3.39 ± 0.28 | 3.38 ± 0.29 | 3.16 ± 0.36 | -6.5% |
| 180 | 3.02 ± 0.35 | 3.02 ± 0.36 | 2.91 ± 0.26 | -3.6% |
| Average = | **3.48 ± 0.67** | **3.47 ± 0.45** | **3.07 ± 0.36** | **-11.5%** |

Values calculated by Registrant from MRID 00122743

*Irrespective of dose group,* pups whose ventricles were dilated tended to weigh less than those without dilated ventricles. Even control and low dose animals (lowest dose tested in this study was the NOAEL) with dilated ventricles weighed less than control and low dose animals without dilated ventricles. This indicates that it is the decreased body weight, not the compound exposure, which results in slightly dilated ventricles. The increased incidence of dilated ventricles at the higher doses was a secondary effect of decreased fetal body weights at these doses, not a direct neurotoxic effect. There is an inverse relationship between pup body weight and incidence of dilated ventricles. As the pup body weight decreases, the likelihood that ventricles may be slightly dilated increases. This is why the incidence of dilated ventricles increased in a dose related manner – because pup body weight decreased in a dose-related manner.

As the number of pups with reduced body weight increased in the higher dose groups, the likelihood that these pups would have dilated ventricles increased. Item 7 under "Toxicology Chapter" further discusses these points.

3. **q1* calculation**: As indicated in previous correspondence, it is the position of BASF that a quantitative low dose extrapolation is not appropriate for acifluorfen. This position will be discussed in several sections that follow. However, even if a q1* were to be calculated, BASF believes that the calculation should result in a value of $1.4 \times 10^{-2}$ (mg/kg bw/day)-1, and not the value of $5.33 \times 10^{-2}$ (mg/kg bw/day)-1 calculated by the Agency.

   A non-genotoxic mechanism of action has been demonstrated for the mouse liver tumors observed with acifluorfen. Therefore, a threshold (MOE) approach should

be used for risk assessment. The Agency has proposed a linear quantitative risk assessment using a q1* calculation. Although this is not considered the appropriate approach, BASF also believes that the dose levels used in the calculation were too high and this led to a q1* value which is too conservative.

The Agency has calculated a q1* of 5.33 X $10^{-2}$ (mg/kg bw/day)-1. This was based upon male mouse liver tumors (adenoma and carcinoma combined). The dose levels used were 0, 29, 62 and 157 mg/kg bw/day with liver tumor incidence values of 9/58, 21/60, 16/56 and 40/59 from control to high dose, respectively. The time to tumor Weibull model was used.

BASF has calculated the q1* value and determined a value of 1.4X $10^{-2}$. The discrepancy between the methods used by BASF and the Agency is in the dose levels. Reported test material intake for males were 119, 259 and 655 mg/kg bw/day. The Agency has reduced the reported dose levels by 24% to correct for the test material being a 24% aqueous solution of acifluorfen, resulting in doses for the q1* assessment of 29, 62 and 157 mg/kg bw/day.

The exact dose levels of acifluorfen in the chronic mouse study (MRID Number 00122732) are, admittedly, somewhat difficult to interpret. The test material supplied to the testing laboratory was acifluorfen acid referred to as “Tackle” or MC10109. The purity of this material was 77% as given in Appendix A of the report. For feeding studies such as this one originally conducted for Rhone-Poulenc, it was decided to test acifluorfen as the sodium salt (the product to be marketed). This was accomplished by mixing the supplied acid with sodium hydroxide in an aqueous solution. This aqueous solution contained 240 g/l of the sodium salt of acifluorfen and was referred to as “Tackle 2S”. The procedure for preparing the aqueous Tackle 2S is found in Appendix E of the report (and reproduced below). The concentration of sodium acifluorfen in the Tackle 2S as well as its stability were determined as part of the study and reported in Appendix A of the report. The aqueous solution was shown to be stable for up to six months at temperatures up to 50°C.

Ingredients for Preparation of Tackle 2S from Powdered Technical MC 10109 (from Appendix E of MRID Number 00122732, Table 1):

For 1 liter acifluorfen-sodium (240 grams/liter):

- 297 grams Technical MC 10109 @ 77% purity = 228.7 grams acifluorfen acid
- 32.9 grams sodium hydroxide
- 1 liter water

Molecular Weight correction factor for acifluorfen acid to acifluorfen sodium =

$$\frac{384.65 \text{ (mol.wt acifluorfen-sodium)}}{361.66 \text{ (mol.wt acifluorfen-acid)}} = 1.06$$

228.7 grams acifluorfen acid x 1.06 = 242 grams acifluorfen-sodium

Final concentration of Tackle 2S solution is 242 grams acifluorfen-sodium/liter

For the preparation of the test diets, the aqueous solution of sodium acifluorfen was then mixed with acetone and added to the feed on a weight/weight basis. Intended test concentrations were 625, 1250 and 2500 ppm. It is difficult from the text portion of the report to determine if these ppm concentrations referred to sodium acifluorfen (Tackle) or the aqueous solution (Tackle 2S). However this is clarified in the test diet preparation SOP (Appendix D). The amount of aqueous Tackle needed to produce the desired test material in feed concentrations was determined with the following equation:

$$\frac{\text{(Concentration in feed [mg/kg] X Quantity of feed [kg])}}{\text{(x) volume of aqueous solution in ml}} = \text{Concentration of aqueous solution (mg/ml)}$$

The concentration of the aqueous solution was 240 mg/ml sodium acifluorfen. So, for example, to prepare 1 kg of the high dose (2500 ppm) feed:

$$\frac{(2500 \text{ mg/kg X } 1 \text{ kg})}{(x) \text{ ml}} = 240 \text{ mg/ml} \qquad (x) = 10.42 \text{ ml}$$

Since the "mg" in the aqueous solution refers to "mg" of sodium acifluorfen, the mg/kg in feed must also refer to sodium acifluorfen. A volume of 10.42 ml of 240 mg/ml solution added to 1 kg gives 2500 mg/kg sodium acifluorfen.

Further support for the dose levels being sodium acifluorfen is given in the analytical verification method in Appendix E of the report (and reproduced below). The 240 g/l aqueous solution was used as the stock solution. From that, two dilutions were made, 1:100 to produce a 2.4 mg/l solution and 1:1000 to produce a 0.24 mg/l solution. The two dilutions were used to prepare standards. For example, to get a standard for 2400 ppm in feed, one ml of 2.4 mg/l stock was added to one gram of feed. This produces a sample with 2.4 mg/g of feed or 2400 ppm. The 2.4 mg/l used refers to 2.4 mg sodium acifluorfen. Therefore the standards reflect levels of sodium acifluorfen and not the diluted aqueous solution.

Standards are prepared by diluting the stock (@240 mg/ml) aqueous solution) to 1:100 (@2.4mg/ml) and 1:1000 (@0.24 mg/ml). The diluted standards are used to spike duplicate 1 gram samples of blank feed in 30 ml centrifuge tubes according to the following scheme:

| **Concentration of stock (mg/ml)** | **Spike added (ml)** | **Feed (g)** | **Concentration of spiked sample (mg/g)** |
|---|---|---|---|
| 2.4 | 2 | 1 | 4.8 |
| 2.4 | 1 | 1 | 2.4 |
| 2.4 | 0.5 | 1 | 1.2 |
| 0.24 | 1.5 | 1 | 0.36 |
| 0.24 | 0.3 | 1 | 0.07 |

When analytical verifications were made, the feed levels were compared against the standards above and reported results reflect actual sodium acifluorfen levels. Therefore, the dose levels as given in the report (625, 1250 and 2500 ppm) should be used as sodium acifluorfen. As given in the Results section of the report, these feed concentrations resulted in average sodium acifluorfen intakes of 119, 259 and 655 mg/kg bw/day from the low to high dose males, respectively. These values should be used for the dose levels for q1* calculations and no adjustment is needed.

When the above higher daily intake values are used, the q1* calculation results in a lower value, 1.4 X $10^{-2}$ (mg/kg bw/day)-1.

4. Pages 3 and 4. References are made to the fact that acifluorfen demonstrated increased susceptibility to offspring in the **rat teratology study**. However, as discussed above under Point 2 and below under "Toxicology Chapter," Point 7, the results of this study and other toxicology studies indicate that the maternal toxicity was likely understated and the developmental toxicity was likely overstated in this study. In addition, the developmental effects are likely secondary to growth delays observed in fetuses, as evidenced by reduced body weights, and do not represent frank developmental toxicity. Rather they are secondary effects due to reduced body weight. Therefore, there is no evidence of increased susceptibility to offspring.

   Based on the lack of susceptibility to offspring, the FQPA safety factor should be removed for both chronic and acute risk assessments.

5. Page 4. Based on the discussion above, the acute population adjusted dose (aPAD) should use only a 100X safety factor, resulting in a value of 0.2 mg/kg/day.

   As discussed in Point 7 under the Toxicology Chapter, a more supportable NOAEL for the chronic population adjusted dose (cPAD) would be 7.5 mg/kg/day from the chronic dog study. No FQPA safety factor is needed, so the cPAD would be 0.08 mg/kg/day.

6. Page 4, **q1* value**. It is BASF's position that a quantitative low dose extrapolation is not appropriate for acifluorfen. A position document has been submitted to the Agency (BASF Reg. Doc. 2001/5000878); a summary of that document is presented in Appendix 1. If a q1* is calculated, there appear to be errors in the Agency's calculation (see Point 3 above).

7. Page 10, Table 2. For the **two-generation reproduction** study the NOAEL for offspring toxicity is given as 1.25 mg/kg/day. The NOAEL in this study should be 50 mg/kg/day as discussed in detail under "Toxicology Chapter" Point 5.

8. Page 11, Table 3. The acute and chronic PAD's should be adjusted as discussed above. The NOAEL used for short-term and intermediate-term dermal exposure should be 300 mg/kg/day from the 21-day dermal toxicity study in rabbits. This is discussed in detail under "Toxicology Chapter" Point 8.

9. Pages 21 and 22, **Dietary Risk from Water**. The Agency states that there may be a risk concern if water exposure is greater that the DWLOC. The only scenario for sodium acifluorfen in which modeled water concentrations exceed the DWLOC is for cancer risk. BASF has recalculated the q1* values (Point 3 above) and the EEC for ground water (discussed in detail in Appendix 4). The Registrant believes, therefore,

that Table 9 entitled "**DWLOCs for Cancer Risk for Acifluorfen**" should be modified as follows:

| Population Subgroup | $Q_1$* | Food Exposure mg/kg/day | Target Max Water Exposure mg/kg/day | Ground Water Herbicide µg/L | Surface Water Herbicide µg/L | DWLOC Cancer µg/L |
|---|---|---|---|---|---|---|
| U.S. Population | $1.4e^{-02}$ | 0.000 | $7.8e^{-05}$ | 0.401 | 1.4 | 2.8 |

**Toxicology Chapter**

1. On page 4, an **Ames assay** using the pre-incubation procedure is requested. This study was recently submitted and been assigned MRID Number 45393902. Additionally, two other Ames assays were submitted with this study and have been assigned MRID Numbers 4532501 and 45393901.

2. On page 5, Table 1, the **acute oral toxicity in rats** is given as 1540 mg/kg for the 40% a.i. This value contradicts the value presented in the "HED Chapter for the Reregistration Eligibility Decision" document (page 7) which gives the rat oral LD50 for the 20.2-23.25% a.i. material as 2025 mg/kg (males) and 1370 mg/kg (females). The latter study is more recent and gives both male and female data. It is suggested that the LD50 data on Tackle (20.2 - 23.25% a.i.) be used consistently in both documents. This would also be consistent with the use of Tackle for the remainder of the acute toxicity testing categories.

3. On page 8, **subchronic toxicity in mice**, the study is classified as "Unacceptable/guideline but upgradeable". No reason is given for the "unacceptable" classification.

4. Pages 10-11, **mouse oncogenicity study** (MRID no. 00122732). It should be added to the review that the high dose in this study (2500 ppm) exceeded the MTD for both male and female animals. In males there was a statistically significant increase in mortality and a body weight decrease of 25% compared to controls at week 79. In females, there was a body weight decrease compared to controls of 34% at week 79. Mortality is certainly an indication that the dosing was too high, and body weight differences of greater than 20% exceed the MTD criteria.

   It should also be noted that the stomach papillomas observed in males and females were significantly increased over control only at the high dose. Although stomach papillomas can be induced in rodents, they are not found in humans because of anatomical differences between the stomach of the human and the rodent. Therefore, the stomach papillomas should not be considered in risk assessments for acifluorfen. This topic is discussed in a position paper that was submitted to the Agency on February 2, 2001 (BASF Reg. Doc. 2001/5000878) and is also discussed in Appendix 2.

   The liver tumors in this study are likely due to a threshold, non-linear MOA. There is evidence that acifluorfen and chemicals of similar chemistry produce liver tumors at high doses via a threshold mechanism of peroxisome proliferation. BASF has presented a position paper on this issue to EPA (BASF Reg. Doc. 2001/5000878). A summary of that petition is presented in Appendix 1.

5. Pages 15 and 16, **Reproductive toxicity in rats**. The doses are described here as being 0, 2.5, 50 and 250 mg/kg/day. In the HIARC report (page13) and the HED RED Chapter (page 10) the doses for this study are described as being 0, 1.25, 25 and 125 mg/kg/day. The Registrant believes the doses as described in the Toxicology Chapter (0, 2.5, 50, and 250) are correct.

   It is stated that in the F2 generation, the incidence of pups dying between lactation days 1 and 4 was significantly increased for the mid and high dose groups when compared to controls. However, the difference from control at the mid dose (500 ppm) is considered spurious and not related to treatment. Pup mortality from day 1 to 4 for the mid dose group was 3.0% compared to the control value of 1.0%. While this increase was statistically significant, the Registrant does not believe that an increase from 1.0 to 3.0% is biologically significant and may only represent random variation. This point of view is supported by the fact that a 3.0% incidence is similar to the test facility historical control data. The LOAEL is also based on dilated renal pelvis at this dose. Pup/litter incidence of dilated renal pelvis (control to high dose): 3/2; 4/4; 8/3; 29/13.

   The registrant encourages the Agency to also consider data from a separate two-generation study that has been submitted to EPA (MRID 00122745). This study used the same strain of rat and same doses of Tackle as the study referred to in the HED Toxicology Chapter (MRID 00155548). Though MRID 00122745 was found to be "Invalid" by the Dynamac reviewers, the information pertaining to day 1 –4 pup mortality and dilated renal pelvis may still be considered scientifically acceptable. The reasons for finding this study to be invalid were that technical grade a.i. was not used and the reproductive performance of the control animals was sub-par. The use of Tackle rather than technical a.i. does not seem to be a valid reason for finding the study invalid since numerous other studies (including the 2-generation study referred to above (MRID 00155548) have been found acceptable by the Agency, and also used either Tackle or Blazer. Furthermore, in these studies, the dose given to the animals was adjusted to account for % a.i. It is true that the control animals had low fertility indexes in this study. Nevertheless, an additional control group was run to increase the sample size of the control animals and, when both control groups are combined, there were 12 litters available to evaluate. There was not an increased incidence of dilated renal pelvis in this study. The pup mortality values for lactation days 0 to 4 in the control and mid-dose (500 ppm) of the F1 and F2 generation from MRID 00122745 are as follows:

   F1 control – 2.8%, F1 Mid-dose – 1.6%
   F2 control - 6.5%, F2 Mid-dose – 3.8%

   (F1 values calculated by Registrant from data contained in Table 19 of the report. The F2 generation in this study consists of a F2a and F2b subgroup. The Registrant has calculated the percent mortality for the combined F2a and F2b generations from Tables 20 and 21, MRID 00122745).

   While the control group "n" value is smaller than may be desired, it is nevertheless evident from examining the data in MRID 00122745, that there is not a treatment-related increase in either dilated renal pelvis or day 0 – 4 mortality at 500 ppm.

   As there were no treatment-related effects in offspring at the mid dose of 500 ppm (50 mg/kg/day) in MRID 00155548, this should be considered the NOAEL for offspring toxicity for this study.

6. Page 17, **Mutagenicity.** The report states that acceptable genetic toxicology studies indicate that sodium acifluorfen was weakly positive in a few assays and negative in the remainder. BASF believes that when all the genotoxicity data collected for acifluorfen are considered, the weight of the evidence indicates that the compound is not genotoxic. BASF has presented a position paper on this issue (BASF Reg. Doc. 2001/5000878) and has also recently submitted three Ames assays (MRID Nos. 45393902, 45323501 and 45393901). Additionally, information requested by the Agency to upgrade MRID 00148272/00122740 (a forward mutation assay at the TK+/- locus in mouse lymphoma cells assay which was negative for genotoxicity) has also been submitted (MRID not assigned).

7. Page 20, **FQPA Considerations/Uncertainty Factor**. The report states that the FQPA 10X safety factor should be retained, due to increased sensitivity of offspring observed in the developmental toxicity study in rats, for acute dietary and short-term/intermediate-term residential (non-occupational) exposures for the Females 13-50, and Infants and Children subgroups. However, BASF believes that the factor should be reduced to 3X for assessing the chronic dietary and long-term residential (non-occupational) exposures for the Females 13-50 and the Infants and Children subgroups.

   The EPA review indicated that increased sensitivity was based upon results from the developmental toxicity study in rats (MRID 00122743). The EPA review states that the NOAEL's for both maternal and developmental toxicity are the same at 20 mg/kg/day; clearly the FQPA factor is being required based not on qualitative susceptibility, but on quantitative susceptibility. Quantitative susceptibility implies that the developmental effects seen at the LOAEL were more severe than the maternal effects. The registrant does not believe there is any evidence that this is the case.

   The EPA executive summary for this study emphasizes the mild nature of the maternal effects at the LOAEL (mid-dose, 90 mg/kg/day). While it is true that the maternal effects at this dose were not severe, it should also be emphasized that the developmental effects at this dose were also very mild.

   The EPA executive summary for the rat developmental toxicity study describes the developmental alterations at this dose as “variations” not malformations. The dilated ventricles seen at this dose were noted in the EPA executive summary for this study to be only “**slightly**” dilated; the dilation of the renal pelvis was noted to be “**slight**” and the delayed ossification was referred to as “**minor**”.

   It is certainly true that the maternal toxicity seen at the mid-dose was not severe by any means. Similarly, the developmental effects seen at the mid dose were also mild.

   Additionally, though the maternal effects certainly are mild, there is evidence that they are somewhat more adverse than the EPA acknowledges. Maternal toxicity was evident at the mid dose of 90 mg/kg/day by clinical signs and a decrease in body weight gain of 7% during the treatment period (the executive summary does not mention this decreased body weight). In addition, a similar dose of 125 mg/kg/day in the 90-day rat feeding study produced hematology effects and liver and kidney toxicity. The same dose of 125 mg/kg/day produced kidney toxicity in the two-generation reproduction study. Had maternal hematology parameters been measured, and the dam’s liver and kidney been examined in the rat developmental toxicity study, it is likely that effects would have been seen at the mid dose.

Furthermore, the pup toxicity observed in the rat developmental study was likely overstated by the EPA. The slightly dilated ventricles and minor delayed ossification are not likely true adverse effects, but are more likely secondary to decreased fetal body weight. Fetal body weights were decreased at the mid and high doses by 9 and 19% compared to controls. Decreases in body weight are often associated with delayed development. This was evidenced in the pups by delayed skeletal ossifications and slight dilation of the lateral ventricles of the brain. At the mid dose of 90 mg/kg/day, the dilation of brain ventricles was observed in 10 pups from 8 litters. The average pup weight from each of these litters was below the control average by 14%. The mean pup weight for those pups with dilated ventricles in all dose groups was 11.5% below the mean pup weight for those pups without dilated ventricles in all dose groups.

Additional information concerning decreased pup body weight in those pups with dilated ventricles compared to those without is shown above under "Health Effects Division Chapter for the Reregistration Eligibility Decision Document" Point 2.

These data demonstrate that these were small pups that would be expected to have developmental delays. The increase in incidence of these developmental delays does not indicate a frank developmental toxicity of the chemical, but an indirect effect of small pups.

In conclusion, the maternal toxicity was likely understated and the developmental toxicity was likely overstated in this study, and (when examined *in toto*) the maternal and developmental toxicity are both mild. Thus, there is no evidence of increased sensitivity for offspring and no need for an additional safety factor. The FQPA safety factor should be removed for both short-term and chronic assessments.

8. Page 21, **Table 2**: **Table of Doses and Toxicological Endpoints Selected for Various Exposure Scenarios**. The chronic dietary non-carcinogenic NOAEL is given as 1.25 mg/kg/day from the two-generation rat reproduction study. As discussed above under Point 5, the NOAEL for this study is 2.5 mg/kg/day, not 1.25 mg/kg/day. However, the NOAEL from the rat chronic/oncogenicity study of 25 mg/kg/day must be considered. As discussed in number 5 above, there were no treatment-related effects on pups at the mid dose of the rat reproduction study of 50 mg/kg/day. However, there was systemic kidney toxicity to the parents at 50 mg/kg/day with the NOAEL being 2.5 mg/kg/day. In the chronic toxicity study in rats, a dose in between these two doses of 25 mg/kg/day was tested without systemic toxicity. Therefore, the overall NOAEL for chronic systemic toxicity in the rat is 25 mg/kg/day.

   **Considering all multiple dose oral studies with acifluorfen, the lowest dose would be 7.5 mg/kg/day in the chronic dog study. This dose should be used for the chronic RfD.**

   The Agency has calculated a q1* value for acifluorfen of $5.33 \times 10^{-2}$ (mg/kg bw/day)-1 based on male mouse liver tumors. BASF believes that incorrect dose levels were used in the calculation and that the actual q1* based on these tumors is $1.4 \times 10^{-2}$ (mg/kg bw/day)-1. BASF has presented a position paper issue (BASF Reg. Doc. 2001/5000878) indicating that a threshold (MOE) approach should be used for cancer risk assessment and that a linear low-dose extrapolation is not appropriate.

   The short-term and intermediate-term dermal NOEL's are from the oral rat teratology study. As indicated in number 7 above, there was no increase in

sensitivity of the young or unborn to acifluorfen. Based on this fact, all studies can be considered. For dermal risk assessment considerations it is more appropriate to use a route to route comparison. The NOEL for systemic toxicity in a 21-day dermal toxicity study in rabbits was 300 mg/kg/day. This should be used for dermal risk assessments.

**Occupational and Non-Occupational Exposure and Risk Assessments**

**Comments on Mixer/Loader and Applicator Exposure and Risk**

1. The current label for the acifluorfen end use product is the APA occupational "Baseline" scenario plus the use of waterproof gloves and eye protection. The "baseline" scenarios described in Appendix B of the HED ORE chapter do not include gloves, while the single layer PPE scenario includes respiratory protection, which is not an acifluorfen label requirement. Thus, a scenario which is equivalent to the label requirements is not included in the HED ORE chapter.

   BASF has recalculated the non-cancer and cancer MOEs for a mixer/loader of liquids for aerial and ground boom application using unit exposure values that match the label requirements. The single layer PPE for maximum application rate absorbed dermal dose values for a mixer/loader of liquids for aerial and ground boom application were taken from Table B4 of Appendix B (single layer was used because it includes waterproof gloves, which the acifluorfen label requires). These values were added to the maximum application rate baseline absorbed inhalation values from Table B3 of Appendix B (the baseline inhalation values were used because this does not include a respirator as per the acifluorfen label). When the NOAEL of 20 mg/kg/day was divided by these values the results were: Non-cancer MOE = 460 for mixer/loader of liquids for aerial application; Non-cancer MOE = 2740 for mixer/loader of liquids for ground boom application. Similar calculations were performed for the cancer risks. The results are presented below:

   Non-cancer mixer/loader liquids for aerial: 20/(0.0345 + 0.009) = **459.7**

   Non-cancer mixer/loader liquids for ground boom: 20/(0.0058 +0.0015) = **2739.7**

   Cancer mixer/loader liquids for aerial:

   $$[(0.0058 + 0.0015) * 20 \text{ days}/365 * 35/70] * 0.053 = \mathbf{4.24 \times 10^{-5}}$$

   Cancer mixer/loader liquids for ground boom:

   $$[(0.0013 + 0.00034) * 20 \text{ days}/365 * 35/70] * 0.053 = \mathbf{9.5 \times 10^{-6}}$$

   These revised risk calculations more accurately reflect the current label for sodium acifluorfen and indicate acceptable risks for mixing/loading liquids.

2. Some of the exposure scenarios are unduly conservative. For example, the probability of an aerial applicator treating 1,200 A/day at the next-to-maximum rate/acre for 30 days/year for a 35-year lifetime is extremely unlikely. The

registrant believes that the burden of showing acceptable risks under conservative assumptions has been met.

**Comments on Residential Exposure and Risk**

3. The residential non-cancer margin of exposure of 4300 reported in Appendix D, Table D2, does not match the non-cancer margin of exposure of 7400 reported on page 26 of the ORE chapter.

4. The inhalation unit exposure value of 2,400 µg/lb a.i. handled shown in Table D1 of Appendix D does not match the PHED value of 1,300 µg/lb a.i. handled. The 2,400 value appears to be taken from the Draft SOPs for Residential Exposure Assessment dated July, 1997. The Final SOPs for Residential Exposure Assessment, dated August of 1998, cite the 1,300 value.

**HED Metabolism Assessment**

1. p. 4. The Agency states that no Canadian tolerances have been established. Sodium acifluorfen is registered for use on soybeans in Canada, with residues of sodium acifluorfen and its metabolites not to exceed 0.1 ppm.

2. p.7. Sodium acifluorfen is classified as a nontranslocated contact herbicide. It enters through the leaves and rapidly acts to destroy cell membranes. Rapid destruction of cell membranes prevents translocation to other regions of the plant.

3. p. 8. The Agency states that if residues of sodium acifluorfen reach groundwater, they will persist indefinitely. Results from an anaerobic aquatic metabolism study (MRID 43155201) have shown that under anaerobic aquatic conditions, the compound is rapidly degraded, with a half-life of approximately 2.7 days.

## PRELIMINARY ECOLOGICAL EFFECTS RISK ASSESSMENT

**Fate and Transport**

1. p.1. In its overview summary document, EPA states that its ability to predict the fate or concentrations of acifluorfen in soil or water has considerable uncertainty and that additional studies are needed to better define the persistence of the compound in the environment. The Agency states:

   **EPA recommends that additional fate studies be conducted for sodium acifluorfen to better understand the fate processes that control its movement in soil under different environmental conditions. Desirable studies include OPP Guidelines 163-1 (Soil Partition Coefficient), 162-1 (Aerobic Soil Metabolism), 162-2 (Anaerobic Soil Metabolism), 162-3 (Anaerobic Aquatic Metabolism), 162-4 (Aerobic Aquatic Metabolism), 164-1 (Terrestrial Field Dissipation), and 164-2 (Aquatic Dissipation).**

   BASF would like to point out that the Agency has stated elsewhere in its review that all environmental fate guideline data requirements for sodium acifluorfen have been

adequately fulfilled. More detailed discussions of the data submitted to fulfill each of these guideline requirements are presented in Appendix 3 and in Appendix 4.

In addition, BASF is submitting with this document literature references concerning the environmental fate of acifluorfen. These literature references address some of the specific concerns cited by the Agency and are consistent with the environmental fate data submitted by BASF. BASF believes that laboratory and field data do present a consistent picture of the fate of the compound in the environment and BASF does not believe that conducting additional studies will add more to this understanding.

In the overview EFED document, under Data Requirements, EPA outlines a set of additional data that it states are "necessary to be able to understand the importance of the different processes under different environmental conditions." BASF is hereby responding to those comments from the Agency.

The Agency states:

**163-1 Soil Partition Coefficient for acifluorfen, amino acifluorfen, and desnitro acifluorfen**

**This study is necessary to support the registration of the herbicide because the sorption of acifluorfen depends upon a number of soil properties. Acifluorfen sorption is also a non-equilibrium, time-dependent process. The mobility of acifluorfen in soil is affected by the rate as well as the maximum extent of sorption. Amino acifluorfen is highly variable, depending upon soil texture. By better understanding the conditions that influence sorption, management options to prevent water contamination would be easier. This information is also needed for subsoils.**

BASF responds:

Two studies were submitted for fulfilling requirements of soil adsorption studies with sodium acifluorfen. One was carried out with acifluorfen acid and one on the acifluorfen amine since this was the only metabolite found at >10% TAR in the environmental fate studies. For the acifluorfen, Koc values for sand, sandy loam, loam, low organic clay and high organic clay soils were 50.22, 73.52, 56.96 198.7 and 168.89, respectively. Adsorption and desorption were strongly correlated with soil clay content. For the acifluorfen amine, Koc values were 431 for sand, 652 for clay, 741 for loam and 7368 for loamy sand, indicating that amino acifluorfen is immobile in loamy sand, of low mobility in loam and clay soil, and of medium mobility in sand soil.

In addition to this worked performed by BASF, the sorptive properties of acifluorfen have been thoroughly examined in recent papers by Gennari et al. (1994) and Locke et al. (1997). **Scientific papers cited in this section and following sections are found in Appendix 4.** In summary, Gennari et al. show that acifluorfen binding in soil is dependent on organic carbon content and soil pH. Destruction of the organic carbon portion of the soils results in lost sorption by the soils. The pH effect was attributed to the net increase in positive charge on the surfaces of iron oxides in soil (not pKa). Since a change in binding was due to the increased charge on the clay portion of the soil, as pH decreased acifluorfen adsorption increased.

Locke et al. (1997) attributed sorption of acifluorfen to organic carbon content, cation exchange capacity (CEC) and soil acidity. Detailed examination of the data indicates that the overwhelming contributor to sorption was made by the soil organic carbon content.

With regard to the primary transformation product of acifluorfen, amino acifluorfen, Andreoni et al. (1994) found that acifluorfen degraded to amino acifluorfen under both oxygen limited and oxygen unlimited conditions. Locke et al. (1997) observed transformation of acifluorfen to amino acifluorfen within 96 hours at 9.9% and 17.8% on Dundee and Sharkey soils respectively. They also looked at the effect of soil temperature on the degradation of acifluorfen indirectly through binding experiments. In their report, they stated that decreased binding was observed in the 4°C treatment compared to the 25C ° treatment. They also observed that the decrease in binding indicated that the transformation of acifluorfen to amino acifluorfen was microbially mediated. It was also noted that amino acifluorfen had a high affinity for soil, which explained the high sorption irreversibility they observed (Kdf 41.3 and 47.2 for Dundee and Sharkey soils respectively). In an additional paper by Gaston et al. (2000), it was determined that intermediate products of acifluorfen were apparently highly sorbed as well.

From the literature it is clear that once degradation of acifluorfen occurs, transformation products adsorb strongly to soil.

The Agency states:

**162-1 Aerobic Soil Metabolism**

**The line between metabolic and chemical degradation of acifluorfen is quite blurry. Acifluorfen also degrades to amino acifluorfen and a number of identified polar substances. The fate of these needs to better characterized.**

BASF responds:

The aerobic soil metabolism of sodium acifluorfen was studied in four soils. The parent slowly declined in all of the soils and the half-lives were estimated to be 100 to 200 days. In the aerobic soil metabolism study parent was the only major metabolite accounting for >10% TAR . The residue declined from 90% TAR at 0 DAT to 43% after six months. The amino and the desnitro analogs were minor metabolites, each accounting for 2.4 to 3.1% TAR at six months. Volatiles accounted for less than 1% TAR.

In addition to this work performed by BASF, a study of the aerobic soil metabolism of sodium acifluorfen has recently been reported in the literature (Gaston et. al., 2000). In this study, the half-life of acifluorfen obtained in the laboratory, using soil columns instead of the standard bioflasks, ranged from 7.2 to 63 days. The results of this study are discussed in detail in Appendix 4.

The Agency states:

**162-2 Anaerobic soil metabolism**

**Acifluorfen is rapidly transformed to amino acifluorfen. The fate of acifluorfen beyond this point is not clear. Its mobility is quite variable, high to low, with apparent persistence.**

BASF responds:

To satisfy the anaerobic soil metabolism data requirements, an anaerobic soil metabolism study was carried out on a clay soil from Mississippi. In this study, acifluorfen rapidly declined from 95.5% TAR at 0 DAT to 6.3% TAR at 10 DAT with a half-life of 2.75 days. The amino analog of acifluorfen was the major metabolite, formed by rapid reduction of the nitro group. It increased from 1.6% TAR at 0 DAT to 76.9% TAR at 10 DAT and then declined to 64.6% TAR at 375 DAT. Acetamide was a minor metabolite found at a maximum concentration of 3.12% TAR at 375 DAT.

In addition, as reported above, recent citations from the literature shed light on the fate of amino acifluorfen under both aerobic and anaerobic conditions. Andreoni et al. (1994) found that acifluorfen degraded to amino acifluorfen under both oxygen unlimited and oxygen limited conditions. Locke et al. (1997) observed transformation of acifluorfen to amino acifluorfen within 96 hours at 9.9% and 17.8% on Dundee and Sharkey soils respectively. They also looked at the effect of soil temperature on the degradation of acifluorfen indirectly through a binding experiment. In their report, they stated that decreased binding was observed in the 4°C treatment compared to the 25C ° treatment. They also observed that the decrease in binding indicated that the transformation of acifluorfen to amino acifluorfen was microbially mediated. It was also noted that amino acifluorfen had a high affinity for soil, which explained the high sorption irreversibility they observed (Kdf 41.3 and 47.2 for Dundee and Sharkey soils respectively). Gaston et al. (2000) determined that intermediate products of acifluorfen were apparently highly sorbed as well.

From the literature it is clear that once degradation of acifluorfen occurs, whether under oxygen limited conditions or oxygen unlimited conditions, transformation products adsorb strongly to soil

The Agency states:

**162-3 Anaerobic Aquatic Metabolism**

**Need to tie into the aerobic conditions better.**

BASF responds:

BASF does not understand the question the Agency is asking. The work of Andreoni et al. demonstrates that the amino compound is formed under both aerobic and anaerobic conditions. The rate of formation may be different depending on the aerobicity of a given system, but that is going to be true for all compounds. BASF can not envision a study that could definitively determine a rate of formation for all states of aerobicity.

The Agency states:

**162-4 Aerobic Aquatic Metabolism**

**The aerobic aquatic half-life suggest that acifluorfen is persistent in an aquatic environment. Aqueous photolysis is quite rapid.**

BASF responds:

An aerobic aquatic metabolism study which was carried out in a clay soil showed acifluorfen to be stable under aerobic aquatic conditions. No other metabolites were detected in this study. The major route of dissipation in water appeared to be by photolysis as shown by the guideline photolysis study where acifluorfen underwent rapid degradation to multiple components, none of which were present at significant concentrations.

The Agency states:

**164-l Terrestrial Field Dissipation**

**This study may be necessary to relate laboratory results to actual field conditions.**

BASF responds:

BASF has carried out a number of studies that examine the behavior of sodium acifluorfen under field conditions. A small scale retrospective study was carried out with the compound (MRID 42152201) in an attempt to answer questions concerning the ability of the compound to leach. Five sites were included in the study. Each site was selected for its representation of a specific growing region (based on soybean, peanut or rice use patterns). Additionally, prior sodium acifluorfen use history (1 to 4 years of prior use) as well as soil vulnerability characteristics were also considered when selecting sites. DRASTIC scores were used to maximize the probability of selecting vulnerable sites. Final site selection was made from an examination of soil type, hydrogeology and annual precipitation information.

Since soil texture, organic matter content, depth to ground water and annual precipitation were used as selection criterion, the sites chosen were extremely vulnerable. By study completion, all sites had received at least three years of sequential sodium acifluorfen applications. Measured rainfall quantities during the conduct of the studies were close to or exceeded historical rainfall amounts. Well water samples were collected monthly for 12 months following the last test substance application. The analytical method used for measuring samples had a LOQ of 1 ug/L. By study completion not a single sample collected during the duration of the study had quantifiable residues of sodium acifluorfen. Results from this study indicates that acifluorfen is not a groundwater concern under conditions of use even on highly vulnerable soils. Additional data collected in this study were field half-lives for the compound at 5 different locations. These were found to be 14 days at the VA site; 22 days at the TN site; 15 days at the NC site; 8 days at the IN site; and 55 days at the ND site.

As noted above, field studies have resulted in half-lives of sodium acifluorfen which range from 8 days (IN) to 55 days (ND). Aerobic soil metabolism studies have given values that range from 108 days (NJ) to 200 days (KS). The Agency has questioned the relationship of field results to laboratory studies, and has stated that a new field study may be required to sort out this discrepancy. Recent results from the

literature provide important clarity to this issue. In a study carried out by Gaston et al. (see Appendix 4), the half-life of acifluorfen obtained in the laboratory using soil columns instead of the standard bioflasks was in excellent agreement with results obtained in field studies. The laboratory degradation data for acifluorfen obtained by Gaston, using the soil column technique, ranged from 7.2 to 63 days. These results are consistent with the data obtained under field conditions, where the measured half-lives ranged from 8 to 55 days.

The Agency states:

**164-2 Aquatic Dissipation**

**The rice studies showed rapid degradation of acifluorfen with low concentrations of degradates formed. The importance of aqueous photolysis (e.g., the rice pond was only 4 inches deep) and reducing (anaerobic processes) conditions is unclear in other water bodies.**

BASF responds:

BASF does not understand the Agency's question. Photolysis will play a role in all aquatic systems where there is exposure to light. Since the rate of photolysis is directly related to light intensity and light intensity is directly related to depth, one presumes that a rate constant for photolysis could be calculated at a given depth. The work of Andreoni et al. demonstrates that amino acifluorfen is formed under both aerobic and anaerobic conditions. The rate of formation may be different depending on the aerobicity of a given system, but that is going to be true over a continuum of environmental conditions and for nearly all compounds which are degraded under both aerobic and anaerobic conditions. BASF can not envision a study that could definitively determine a rate of formation for all states of aerobicity.

Based on the discussion above, BASF believes that the data required to understand the behavior of sodium acifluoren in soil and water under different environmental conditions exist. The Agency has stated in its review that all environmental fate guideline requirements for sodium acifluorfen have been fulfilled and BASF does not believe that repeating any of these studies will allow a better definition of the variability of the persistence or mobility of the compound in the environment.

**USE CHARACTERIZATION**

1. p.5. Use Characterization: BASF would like to point out that these marketing data do not reflect the current reality of the agricultural marketplace. Due to the impact of biotechnology, especially in the soybean market, the acreage estimates for sodium acifluorfen-containing products have been significantly reduced over the past two years. BASF presents a more current quantitative use assessment in Appendix 5; this information is considered to be Confidential Business Information.

2. In addition, in Appendix 6, BASF presents the benefits of sodium-acifluorfen-containing products when compared to those potential alternatives listed in the EPA overview document of the preliminary risk assessment.

**ECOLOGICAL EFFECTS**

1. p. 35. EPA states that it has performed a screening level assessment and a higher tier modeling assessment of the risk that acifluorfen may pose to birds and concludes that there may be a chronic risk to birds that eat short grass and to insects after the use of the compound on peanuts, soybeans, or rice.

   The assumptions used by EPA in their assessments are quite conservative and certainly overstate the potential risk of the compound. Some examples of the conservative nature of the assessment are presented below.

   First, EPA assumes a 30-day half-life for residues in short grass or insects. This value is extremely conservative. Acifluorfen is a contact herbicide that is not systemic and is not translocated throughout the plant. Consequently, BASF believes that the primary potential route of exposure to wildlife will be residue on the leaves of plants. BASF has submitted a study (MRID 44091101) entitled "Foliar Dislodgable Residues of Blazer on Soybeans" which reports a half-life of less than one day on the treated plants and no detectable residues after 3-5 days. Admittedly, the study was designed as dislodgable study and not a foliar dissipation study. However, BASF believes that the study provides compelling evidence that the foliar half-life of acifluorfen is significantly less than 30 days and, therefore, contends that exposure, and thereby chronic risk, to birds will be minimal.

   Additionally, guidance being developed by experts from the EU states that conservative half-lives on many matrices is 7 days. Exposure modeling, as done in Appendix B of the EPA assessment, using a more realistic half-life of 7 days will show that predicted residue levels exceed the avian NOAEC for only a few days. It should also be noted that the EPA has data in hand showing that the photolytic half-life of acifluorfen is about 3.8 days. Thus, a half-life of 7 days on the surface of leaves is not unreasonable.

   Finally, EPA assumes that birds will consume 100% of their diet from treated fields. Acifluorfen is applied at early post-emergence when fields will not have lush stands of short grass or high populations of insects. Therefore the chosen avian food items are of limited relevance to actual avian risk. Large stands of short grass and populations of insects may be present adjacent to treated fields. However, these would only be exposed to the compound by drift and residue levels would be a small fraction of the in-field values predicted by Kenaga. In its probabilistic assessment for ChemX, EPA acknowledged that birds do not spend 100% of their time foraging in agricultural fields. Although the proportion of the diet that birds do obtain from agricultural fields varies, EPA estimated values from 10% to 76% for birds in corn for ChemX.

   In summary, the assessment performed by EPA is very conservative and does not assure that a true risk to birds will occur in the field. A more realistic assessment that includes a 7-day half-life and a smaller fraction of the diet obtained from the treated field would result in Risk Quotients below EPA's Level of Concern.

2. p.1. The Agency states that it is uncertain about the chronic risk of acifluorfen to freshwater and estuarine animals. However, based on the data set submitted and reviewed for acifluorfen, EPA, in Chapter 3 of the preliminary risk assesment ("Integrated Environmental Risk Characterization"), clearly states that no chronic

risk for freshwater and estuarine animals is anticipated. However, in Chapter 5, EPA presents some concerns based on effects seen in a fish early life stage study conducted with a different compound, carfentrazone-ethyl. Since BASF is not able to review the data for the carfentrazone-ethyl study and is unaware of the similarities between the two compounds, we do not feel we can comment on EPA speculation that these two compounds have similar toxicity profiles.

The Agency states that a preliminary phytotoxicity with carfentrazone-ethyl showed a nearly 20-fold increase in toxicity when natural wavelength light was used. Since guideline studies conducted in 1982 did not use natural wavelength light, the assumption is stated that a similar increase in toxicity may be seen with acifluorfen. While the available BASF study does not provide a clear NOAEC, of all the endpoints measured, the only endpoint statistically different than the control at 1.5 mg/L was weight. Other endpoints measured during the 36-day continuous exposure included egg mortality, fry mortality, hatchability, survival, and length. For acifluorfen, a 20-fold increase in toxicity would result in a potential chronic effect level at 75 µg/L. Even at this level of toxicity, a comparison of the 60-day average EEC for soybean and peanuts (approximately 20 µg/L) and the 56-day average for rice (approximately 8 µg/L) to the lowest level tested (1.5 µg/L) does not result in a chronic risk exceedence. The RQ for all crops would be less than 1 and LOCs would not be exceeded.

It should also be noted that no fathead minnow early life stage study is required for acifluorfen because 1) none of the criteria were exceeded except reproductive impairment in birds and, 2) the 60-day PRZM/EXAMS value is less than 20 µg/L, which is below 1/100 of the lowest LC50 (17 ppm).

3. p.1. The Agency states that it is uncertain about the risk of acifluorfen to non-target terrestrial plants. BASF agrees that, based on the studies provided, a risk assessment for non-target terrestrial plants would be limited. BASF is willing to work with the Agency to further define the risk.

**DRINKING WATER EXPOSURE ASSESSMENT**

1. p. 20. **Water Resources Assessment**: The Agency states that it believes that the major risk associated with the use of sodium acifluorfen is the contamination of surface and ground water. EPA has used the SCI-GROW model to estimate potential ground water concentrations for acifluorfen and acifluorfen as a degradate of lactofen under hydrologically vulnerable conditions. BASF does not agree with the Agency's use of certain values in the SCI-GROW calculations and has data that contradict certain of the values that were used in the modeling exercise. BASF presents a detailed response to these evaluations in Appendix 4.

   Based on the data presented in Appendix 4, BASF has calculated a revised worst case SCI-GROW Ground Water Screening Concentration of 0.401 ppb. Therefore, the modeled concentrations of sodium acifluorfen in ground water would be recalculated as:

   Target Maximum Water Exposure (mg/kg/day): $7.8 \times 10^{-5}$

   EEC of Sodium Acifluorfen (Ground Water): 0.401 µgm/l

EEC of Sodium Acifluorfen (Surface Water): 1.4 µgm/l

Total EEC for Surface and Ground Water: 1.801 µgm/l

The DWLOC for cancer risk for sodium acifluorfen has been recalculated by BASF (see page 6 of this document) to be 2.8 µgm/l. Therefore, modeled water concentrations do not exceed the DWLOC and the modeled water concentration is below the Agency's level of concern.

**PRODUCT AND RESIDUE CHEMISTRY CHAPTERS**

**Product Chemistry**

1. p. 3. On page 3, EPA discusses the regulatory history of sodium acifluorfen as it pertains to the product chemistry which supports currently registered sodium acifluorfen products. The discussion is slightly in error. In actuality, Rohm and Haas Company was the first registrant of sodium acifluorfen. This first registration was granted for the Rohm and Haas product Blazer herbicide in 1980. In 1987, BASF purchased the registration and data that supported that product. BASF contracted for the toll manufacture of the active ingredient at the Rohm and Haas facility in Bayport, Texas. Rohm and Haas has continued to toll manufacture the active ingredient for BASF under the Rohm and Haas process since the purchase and continues to produce sodium acifluorfen using that original manufacturing process. Under the requirements of PR Notice 87-7, BASF registered the sodium acifluorfen MUP that is produced at the Rohm and Haas facility so that product could be moved from Bayport, Texas to various BASF formulating facilities.

   In 1984, Rhone-Poulenc registered its own sodium acifluorfen product, Tackle. Rhone-Poulenc used a slightly different manufacturing process; material was produced in a separate facility in Tennessee. In 1992, Rhone-Poulenc relinquished its sodium acifluorfen business and sold its database for sodium acifluorfen to BASF. Rhone-Poulenc no longer maintains any registrations for Tackle.

   The product chemistry database that BASF has submitted to EPA under the requirements of FIFRA '88, and that EPA has found to be acceptable, has been generated for material produced in the Rohm and Haas production facility.

2. p. 2. Bulk density packed should be 32.08 lb/ft$^3$ (packed).

3. p. 6. 830.1750 Certified Limits. This study is required for a TGAI. The submitted study has been assigned MRID 41891203.

4. p. 6. 830.1800 Enforcement Analytical Method. This study is required for a TGAI; the submitted study was assigned MRID 41891202.

**Residue Chemistry**

1. p. 11. BASF currently maintains registration for 6 end use sodium acifluorfen products. A sixth product, Conclude Xact (EPA Reg. No. 7969-179), was registered by EPA on March 29, 2000.

2. p. 16. Under "Residue Analytical Methods," the Agency suggests that the diazomethane used in the analytical method be replaced with an alternative methylating reagent. BASF has investigated numerous other methylating reagents (e.g., methanolic HCl) in this analytical procedure. However, only the diazomethane method produced satisfactory and reproducible results. In addition, the diazomethane is used as a dilute, ethereal solution. BASF recommends using the ethereal diazomethane method while employing standard safety practices to prevent safety incidents.

3. p. 16. Under "Method for determination of residues...", the Agency states that no radiovalidation data have been submitted for the enforcement method (PAM II), and that these remain outstanding. BASF believes that the radiovalidation experiments are of little value based on the low residue situation that exists for sodium acifluorfen in seeds or grains. Residues of concern in the metabolism studies are at or below the limits of quantitation for the final analytes. BASF believes that the nature of the extraction scheme in the enforcement method is chemically reasonable for releasing any residues of concern. The metabolism studies have shown good extractability of the residues of concern in organic solvents such as methanol. The acetonitrile/aqueous acidic extraction techniques involved in the enforcement method are expected to be at least as efficient if not more so, compared to the metabolism extraction scheme. BASF believes radiovalidation would produce at the best marginal data because of the low residue levels.

4. p. 17. In the paragraph that continues from page 16, the Agency states that the validated limit of quantitation is 2.05 ppm for rice straw (0.05 ppm for acifluorfen and acifluorfen methyl ester and 2.0 ppm for acifluorfen amine and its methyl ester). The agency in addition states that this LOQ is above the level determined in the rice straw (<0.124 ppm). BASF disagrees with the claim of 2.0 ppm as the LOQ for acifluorfen amine and its methyl ester. In the method validation report (MRID 44153801), it was shown that recoveries for acifluorfen, its methyl ester, acifluorfen amine, and its methyl ester, were acceptable at the 0.05 ppm level. The recoveries for the amine metabolite were lower than the other compounds at 55±9% (n=8), but the precision was good with a standard deviation below 10%. In addition, during the analyses of the crop field trial straw samples (MRID 43584502), concurrent recoveries of the amine metabolite at levels of 0.05 and 0.2 ppm ranged from 70-80% (five recovery samples run in total). BASF believes the limit of quantitation of 0.05 ppm for each analyte is appropriate.

5. p. 17. In a discussion of independent laboratory validation, the Agency states that radiovalidation data must be submitted before the method (D9205) can be considered acceptable for tolerance enforcement purposes. BASF is satisfied with having the current PAM II method used as the enforcement method. BASF also considers the extraction procedure in D9205 to be more exhaustive than the enforcement method, and thus has not confirmed the method by radiovalidation. The enforcement method uses an acetonitrile/acidic aqueous solvent for extraction.

The data collection method first uses an aqueous basic soak followed by an acetonitrile/acidic aqueous solvent.

6. p. 21. In the discussion of the confined rotational crop study, EPA states that 14C-residues >0.1 ppm accumulated in/on all rotational crop commodities of chard, turnip, sorghum, wheat, and radish planted 39, 103, 145, 313, and/or 370 days following application. BASF believes that a typographical error was made, that the value should be 0.01 ppm, and that the statement should read "that 14C-residues >0.01 ppm accumulated in/on all rotational crop commodities of chard, turnip, sorghum, wheat, and radish planted 39, 103, 145, 313, and/or 370 days following application."

7. p. 22. The conclusion of this section states that based on the results of the confined rotational crop study (MRID 42785601), the labels for sodium aciflurofen should be amended to specify a 12-month plant back interval (PBI) for rotated crops, while a 6-month PBI would be acceptable for small grain crops. BASF does not agree that the labels should be amended to specify a 12-month plant back interval for rotated crops with a 6-month PBI allowed for small grain crops. BASF's opinion is based on the following considerations: Although total radioactive residues were found to be greater than 0.01 ppm for most of the samples, the individual residues of concern appear at a much lower concentration and would not be detectable with the current analytical methodology. In both the enforcement and data collection methodologies, residues of sodium acifluorfen, which include the acid and salt version of acifluorfen, the methyl ester of acifluorfen, the amine metabolite and its methyl ester, are determined as a combination of two final analytes. In the enforcement method, all residues of concern are converted to either the methyl ester of acifluorfen or the heptafluorobutyric amide equivalent of the amine metabolite. In the data collection method, all residues of concern are converted to either the methyl ester of acifluorfen or the amine metabolite. The collective limit of quantitation (LOQ) for the final analytes sums to 0.1 ppm, 0.05 ppm per analyte. Because no quantifiable residues of acifluorfen are seen in most crop matrices (rice grain, peanut nutmeat, and soybeans), tolerances have been set at the 0.1 ppm LOQ value. Based on either the enforcement method or the residue data generation methods, residues of acifluorfen would not be measurable. The only residue of concern identified in the confined study was acifluorfen, and this component never exceeded 0.024 ppm, even at the 39 day emergency plant back interval. This value is well below the 0.1 ppm tolerance, which is based on the methodology LOQ. Based on this information, BASF feels that no plant back restrictions based on the residue situation should exist for sodium acifluorfen.

BASF is hereby submitting two paper copies of this document, one version which contains an appendix (Appendix 5) that includes information claimed as Confidential Business Information (CBI) and one version that does not contain this information. We are also submitting an electronic copy of this document (Adobe format) which does not contain the CBI information. If you have any questions or comments on this submission, please contact me at (919) 547-2979.

Sincerely,
BASF Corporation
Agricultural Products

Karen R. Blundell
Registration Scientist

**APPENDIX 1: SUMMARY FROM A PETITION ENTITILED "SODIUM ACIFLUORFEN: REQUEST FOR REASSESSMENT OF CURRENT CANCER CLASSIFICATION CONSIDERING PEROXISOME PROLIFERATION-ASSOCIATED MODE OF ACTION" (MRID NUMBER 2001/5000878**

**APPENDIX 1: SUMMARY FROM A PETITION ENTITILED "SODIUM ACIFLUORFEN: REQUEST FOR REASSESSMENT OF CURRENT CANCER CLASSIFICATION CONSIDERING PEROXISOME PROLIFERATION-ASSOCIATED MODE OF ACTION" (MRID NUMBER 2001/5000878)**

BASF respectfully petitions the EPA to reconsider the current cancer classification of acifluorfen based on the facts that:

- A Weight-of-the-Evidence evaluation of the mutagenicity data for the compound indicates that acifluorfen is not mutagenic.
- There are data indicating that acifluorfen posseses peroxisome proliferation activity.
- A mode of action for the liver tumors in mice, based on increased hepatic cell proliferation, has been demonstrated.
- The stomach tumors seen in the B6C3F1 mouse are of a type never seen in humans and are not reproducible in other rodent bioassays.
- EPA's proposed cancer guidelines state that if a compound which is associated with cancer occurrence in rodent studies can be shown to be non- mutagenic, and a mode of action for the carcinogenic effects can be demonstrated, then a non-linear, mode of action-based cancer risk assessment may be performed.

As evidence for the points listed above, we will show that:

1. The weight of the evidence from an examination of the mutagenicity database indicates that acifluorfen is not mutagenic. Fourteen out of nineteen submitted genotoxicity/mutagencity studies were negative. Three of the five positive studies were only weakly positive and were described by EPA reviewers and the study authors as such.

2. Acifluorfen exposure through the diet clearly increases the incidence of hepatocellular tumors in the B6C3F1 strain of mouse, but not in the CD-1 mouse or F-344 rat. A mode of action has been established for these hepatocellular tumors. This mode of action involves an increase in cell proliferation which renders the liver susceptible to tumor formation. Increased hepatic cell proliferation following acifluorfen exposure is evidenced by observations of hepatomegaly and histopathology findings, and, the positive results of an *in vivo-in vitro* assay .

   Associated with this mode of action is a general scientific consensus that increased hepatic cell proliferation has been proposed as being one of the ways in which peroxisome proliferators induce hepatic carcinogenesis.

Acifluorfen displays many characteristics seen in compounds known to be peroxisome proliferators. Acifluorfen contains structural features representative of compounds known to be peroxisome proliferators (*i.e.* – a terminal carboxyl group, ether linkage, lipophillic nature). Acifluorfen exposure increases the activity of palmityl-CoA oxidase . Also, lactofen, a compound which is metabolized primarily to acifluorfen, increases the levels of hepatic catalase and palmityl and acyl-CoA oxidase exposure in rats following *in vivo* exposure, and increases the number of peroxisomes in primary rat hepatocytes following *in vitro* exposure.

The property of acifluorfen which constitutes its proposed mode of action – its ability to induce increased hepatocellular proliferation – has been found to have a no observed effect level. Increased hepatocellular proliferation was not seen at doses at or below 320 ppm (50 mg/kg/day in males and 65 mg/kg/day in females) in a 90-day study in the B6C3F1 mouse. Likewise, an increase in liver weight was not seen at 188 ppm (29 mg/kg/day in males, 38 mg/kg/day in females) in a 4 week study using this strain of mouse, but was seen at 522 ppm (82 mg/kg/day in males, 107 mg/kg/day in females).

**The NOEL proposed for an acifluorfen MOE-based cancer risk assessment is 50 mg/kg/day, based on induction at the next highest dose of hepatic cell proliferation in male mice in a 90-day bioassay. An additional safety factor of 3 may be considered to account for the use of a subchronic study in determining a chronic NOEL.**

3. The stomach tumors seen following acifluorfen exposure were seen in a single strain of mouse (B6C3F1) and were not seen in the CD-1 strain or in F-344 rats. The tumors seen were papillomas – a tumor type that *never* occurs in the stomachs of humans. The site of occurrence was the forestomach – a portion of the rodent stomach for which there is no human equivalent.

Because of the type of tumor observed and the strain specificity of the oncogenic response, the human relevance of the observation of stomach papillomas in B6C3F1 mice following acifluorfen exposure is likely nil.

**Conclusion:**

The weight of evidence indicates that acifluorfen is not mutagenic.

There is ample evidence demonstrating that acifluorfen exposure to the B6C3F1 mouse induces hepatocellular proliferation and that this proliferation may be associated with hepatic tumor formation.

Stomach tumors seen after acifluorfen exposure are seen in only one strain of mouse and occur in an area of the rodent's stomach known to be susceptible to chemical-induced carcinogenesis and for which there is no human equivalent.

The lack of mutagenicity, demonstrated mode of action for the liver tumors, and lack of reproducibility of the stomach tumors in CD-1 mice or F-344 rats, all indicate that a cancer risk assessment for acifluorfen is most appropriately conducted using a nonlinear, margin of exposure (MOE) approach.

# APPENDIX 2: SODIUM ACIFLUORFEN- INCIDENCE OF BENIGN STOMACH PAPILLOMAS IN B6C3F1 MICE

**APPENDIX 2: SODIUM ACIFLUORFEN- INCIDENCE OF BENIGN STOMACH PAPILLOMAS IN B6C3F1 MICE**

Acifluorfen exposure has been shown to result in an increased incidence of stomach tumors in B6C3F1 mice. These tumors are papillomas – a benign tumor derived from stratified epithelial cells.

## Incidence of Stomach Papillomas in B6C3F1 Mice (MRID 00122732)

| **Males** | | | | |
|---|---|---|---|---|
| | 0 | 625 ppm | 1250 ppm | 2500 ppm |
| Papillomas | 0/49** | 0/46 | 0/43 | 4/40 (10)* |
| Papilloma historical control data[1] | | | Mean = 0.2% | Range = 0%- 1.4% |
| **Females** | | | | |
| | 0 | 625 ppm | 1250 ppm | 2500 ppm |
| Papillomas | 0/45* | 3/48 (6) | 4/47 (9) | 6/45 (13)* |
| Papilloma historical control data[1] | | | Mean = 0.5% | Range = 0%- 4.3% |

Values in parenthesis are percent

* $p < 0.05$. ** $p < 0.01$. Significance for trend is shown in control column.
[1] Historical control data from Charles River Labs, February, 1990

These stomach papillomas were found to occur in the forestomach, or, nonglandular, portion of the stomach. The rodent forestomach comprises about 50% of the stomach in the mouse and about 60% in the rat (Kaplan, *et al*., 1983; Brown and Hardisty, 1990). The upper portion (proximal to the esophagus) of the stomach is the forestomach while the glandular portion (comprised of a fundus and pylorus) comprises the lower portion. The epithelium of the rodent forestomach is a stratified squamous epithelium, very much like the epithelium of the rodent esophagus. Papillomas, by definition, arise from stratified epithelium. Humans do not have a nonglandular portion in their stomach. The entire human stomach is glandular – similar to the lower half of the rodent stomach. Because the human stomach lacks a stratified sqamous epithelium, stomach papillomas are unheard of in man.

Though unheard of in humans, forestomach tumors are readily induced by chemical exposure in rodents. Huff, *et al*., found that forestomach tumors were the third most commonly occurring tumor in both male and female mice in cancer bioassays from the NCI/NTP database (Huff, *et al*., 1991). This same data showed that forestomach tumors were the fourth most common tumors in male rats and the fifth most common tumors in female rats (the strains most often used in NTP cancer bioassays are B6C3F1 and F-344).

The foremost characteristic of the rodent forestomach which makes it so susceptible to chemical carcinogenesis is that of exposure. The forestomach of the rodent has been described as "a bag that holds food before it passes through the stomach into the intestinal tract" (Clayson, *et al*., 1990). A bolus of ingested food can be stored in the forestomach for between 12 and 16 hours ( Brown and Hardisty, 1990). Clearly, the exposure of the forestomach to food-borne xenobiotics is extensive given this situation. A comparison of the rodent forestomach to the rodent esophagus is useful in this respect. The rodent esophagus is anatomically located adjacent to the forestomach, is comprised of the same type of epithelium, and is exposed to the same food-borne xenobiotics as the forestomach. However, ingesta passes rapidly through the esophagus. Esophageal tumors are very rare in rodents. In fact, literature surveys conducted in the late 1980's, found only one published incidence of chemically-induced esophageal tumors in rats, and none in mice (Grice, 1988).

Though chemical-induced stomach tumors are quite common in rodents, acifluorfen failed to induce stomach tumors in two out of three rodent bioassays. No stomach tumor of any type was seen in either sex at any dose in previously described 24 month bioassays with CD-1 mice (MRID 00082897) or F-344 rats (MRID 00128253).

Summary - Acifluorfen appeared to be associated with an increased incidence of stomach papillomas in B6C3F1 mice. This tumor type is one which does not occur in humans and occurs in a tissue type (non-glandular portion of the stomach) which is not present in humans. It is very important to note also, that the tumors seen in the B6C3F1 mouse were not reproducible in two other rodent bioassays using the F-344 rat and the CD-1 mouse. Given this, it seems likely that the observation of an increased incidence of forestomach papillomas in the B6C3F1 mouse following acifluorfen exposure does not have relevance to humans.

# APPENDIX 3: REVIEW OF LABORATORY ENVIRONMENTAL FATE STUDIES FOR SODIUM ACIFLUORFEN

## APPENDIX 3: REVIEW OF LABORATORY ENVIRONMENTAL FATE STUDIES FOR SODIUM ACIFLUORFEN

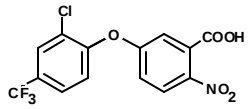
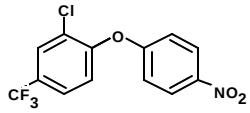
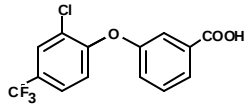
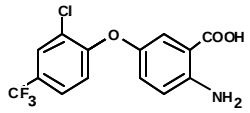
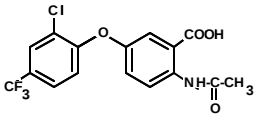
### Assessment of Laboratory Environmental Fate Studies

EPA has determined that the guideline studies to support the environmental fate assessment for sodium acifluorfen are acceptable. From the laboratory data perspective this includes studies which examine hydrolysis; photodegradation in water and soil; aerobic and anaerobic soil metabolism; aerobic and anaerobic aquatic metabolism; and leaching and adsorption / desorption of parent compound and degradates.

The available data indicate that the major routes of dissipation are via chemical and microbial mediated processes. Sodium acifluorfen rapidly undergoes photolytic degradation in water, but is photolytically stable on soil. It is stable to hydrolysis. Aerobic soil metabolism proceeds moderately with half-lives ranging from 108 to 200 days in bioflask studies performed according to current guidelines, whereas under anaerobic conditions, reduction of sodium acifluorfen to the amino analog is rapid, resulting in a half life of 2.75 days.

The acifluorfen free acid has a very low affinity for adsorption. The $K_{oc}$ values for sand, sandy loam, loam, low organic clay and high organic clay soils were 50.2, 73.5, 57.0, 198.7, and 164.9, respectively. Acifluorfen amine, on the other hand, has a much higher affinity for binding to all of the soil types tested. The $K_{oc}$ values were 431, 652, 741, and 7368, for sand, clay, loam, and loamy sand, respectively.

**The Structures of Acifluorfen and Its Metabolites.**

| Structure | Abreviation(s) | Chemical Name |
|---|---|---|
|  | Acifluorfen | 5-[2-chloro-4-(trifluoro-methyl)phenoxy]-2-nitro-benzoic acid |
|  | Descarboxy acifluorfen | 4-[2-chloro-4-(trifluoro-methyl)phenoxy]-nitrobenzene |
|  | Desnitro acifluorfen | 3-[2-chloro-4-(trifluoro-methyl)phenoxy]-benzoic acid |
|  | Amino acifluorfen | 5-[2-chloro-4-(trifluoro-methyl)phenoxy]-2-amino-benzoic acid |
|  | Acetamide | 5-[2-chloro-4-(trifluoro-methyl)phenoxy]-2-acetamido-benzoic acid |

Environmental Chemistry, Fate and Transport

## Hydrolysis (GDLN 161-1)

$^{14}$C-sodium acifluorfen was stable to hydrolysis in pH 4.5, 7.2 and 9.7 buffer solutions.at 25 °C and at two concentrations of the active ingredient (1 and 50 ppm).

Reference:

Keene, E. 1991. BASF Corporation Phase 3 Summary of MRID 00107479: A Hydrolysis study with $^{14}$C-RH-6201 in Water. Accession No. 095735, BASF Registration Doc. No. 75/5009, MRID # 92168032.

Concentration of $^{14}$C-Acifluorfen (Labeled in the Carboxyl Group) in the Hydrolysis Solutions at Various Temperatures and pH. Data Compiled from MRID #92168032.

| DAT | Conc. (PPM) | | 25°C | | | 36°C | | | 48°C | |
|---|---|---|---|---|---|---|---|---|---|---|
| | pH | 4.5 | 7.2 | 9.7 | 4.5 | 7.2 | 9.7 | 4.5 | 7.2 | 9.7 |
| 0 | 50 | 46.82 | 48.87 | 49.12 | 46.43 | 48.72 | 49.19 | 47.09 | 48.30 | 49.00 |
| 7 | 50 | 50.77 | 49.61 | 49.19 | 49.84 | 50.95 | 49.07 | 51.11 | 48.79 | 49.49 |
| 14 | 50 | 57.61 | 55.87 | 53.03 | 53.07 | 55.96 | 57.36 | 56.23 | 56.23 | 56.22 |
| 28 | 50 | 50.90 | 50.63 | 49.18 | 51.75 | 52.10 | 53.02 | 53.71 | 52.92 | 54.27 |
| 56 | 50 | 53.45 | 53.14 | 51.43 | 54.99 | 55.16 | 54.81 | 59.96 | 55.80 | 59.89 |
| 0 | 1 | 1.04 | 1.06 | 0.95 | 0.97 | 1.02 | 1.01 | 1.00 | 0.99 | |
| 7 | 1 | 1.11 | 1.14 | 1.12 | 1.03 | 1.04 | 1.11 | 1.10 | 1.09 | 1.10 |
| 14 | 1 | 1.26 | 1.26 | 1.07 | 1.16 | 1.21 | 1.18 | 1.01 | 1.27 | 1.26 |
| 28 | 1 | 1.09 | 1.12 | 1.12 | 1.10 | 1.18 | 1.14 | 1.22 | 1.17 | 1.68 |

## Photodegradation in Water (GDLN 161-2)

An aqueous photolysis study (BASF Study # M9118, MRID # 41891208) was submitted to the EPA in May of 1991 and reviewed in December 1991. This study was not accepted by the Agency because only one of the two phenol rings (F-label) was used and, according to the reviewer, 50% of the radioactivity described as polar material went uncharacterized and unquantitated. However, the study showed the ethyl acetate extract of the photolysis mixture to be a complex mixture of photolytic degradative products in which no single product constituted more than 10% of the initial photolysis solution.

Therefore, a new aqueous photolysis study was conducted using both N and the F-label acifluorfen (BASF Report # M9311 MRID # 42793502). This study also showed the photolytic degradative products to be a complex mixture of products consisting of one major polar (24 to 35%) and 4 minor polar products (2 to 8%) other than parent by HPLC analysis. The major polar product was shown to be a complex mixture of components. The half-lives for the N and the F-labels were shown to be between 78 to 94 hours. The mass spectral analysis of the reaction products was inconclusive. The basic conclusion from these studies was that acifluorfen rapidly undergoes photolytic degradation in water to a complex mixture of components, none of which is abundant enough to be a concern regarding environmental residues.

A supplementary study (BASF Report # M9314, MRID # 41891208) was carried out to provide additional evidence that photolysis leads to numerous polar photoproducts, none of which are formed in significant amounts. For this study, the photolysis samples from the first study were reanalyzed by 2D TLC and ion-pair chromatography. These additional analyses also showed that the photolytic degradative products were a complex mixture of components none of which exceeded 6.5% of the total applied radioactivity.

An additional study was carried out with $^{14}$C-acifluorfen (N-label) at a concentration of 102 and 21 ppm for 0 to 360 hours of continuous illumination at a light intensity of 1800 uEinsteins-m2s-1 at 25°C. The study also examined photolysis of $^{14}$C-aciflourfen (F-label) for 360 hours and compared it to the photolysis products produced by the N-label. The major degradation product was $CO_2$ reaching a maximum of 7% TAR in 102 ppm series and 10.4% TAR in 21 ppm series. The half-life of acifluorfen was estimated to be 352 and 298 hours in the 102 and 21-ppm series, respectively. The parent concentration declined to 38 to 46% with an increase in polar peak to 31 to 38% TAR after 360 hours of continuous irradiation. Extensive HPLC and LC/MS showed polar peaks to consist of multiple components with twelve non-distinct peaks with concentrations ranging from 0.55 to 4.83% TAR; parent peak was unaffected by the same techniques. Photolysis of $CF_3$-labeled acifluorfen yielded similar reaction products and there was no ring cleavage under aqueous photolysis conditions. The results of the new study are similar to the previous reports showing only the presence of a polar peak and parent peak in the irradiated solutions. As reported in the previous studies, the polar product consisted of

multiple components none of which were present at significant concentrations to be of major environmental concern.

References:

Panek, M. 1991. Aqueous Photolysis of $^{14}$C-Sodium Acifluorfen". BASF Report # M9118, MRID # 41891208.

Panek, M. 1993. Aqueous Photolysis of $^{14}$C-Sodium Acifluorfen: Supplementary report to MRID # 41891208, MRID # 44195001.

Suter, P. 1993. Artificial Sunlight Photolysis of Acifluorfen in Aqueous Media at pH 7.0. BASF Report # M9311, MRID # 42793502.

Venkatesh, K. 1996. Further Characterization of Photolytic Degradation Products from the $^{14}$C-Acifluorfen [$NO_2$ Label] Artificial Sunlight Photolysis. BASF. Reg. Doc. No. 96/5112. MRID # 44195002.

Distribution of Radioactivity in various Fractions in the Aqueous Photolysis Experiments Conducted with $^{14}$C-Aciflurofen (N-Label) at 102 ppm and 21 ppm Concentrations. Data Compiled from MRID # 44195002.

| Irradiation Time (Hr) and Concentration | % TAR in | | | |
|---|---|---|---|---|
| | HPLC Analysis HPLC Analysis of Photolysis Solution | | | |
| | Polar | Parent | Others | $CO_2$ |
| 102 PPM | 0.00 | 97.90 | 2.10 | 0.00 |
| 0 | 1.68 | 96.22 | 1.47 | 0.00 |
| 20 | 3.24 | 89.43 | 3.42 | 0.02 |
| 40 | 5.34 | 87.12 | 5.25 | 0.00 |
| 72 | 10.17 | 77.47 | 6.42 | 0.65 |
| 120 | 11.72 | 77.36 | 6.10 | 0.49 |
| 168 | 21.24 | 62.40 | 7.11 | 2.30 |
| 288 | 25.42 | 59.14 | 6.39 | 3.51 |
| 360 | 30.71 | 46.12 | 7.64 | 6.79 |
| | | | | |
| 21 ppm | | | | |
| 0 | 0.00 | 98.48 | 1.52 | 0.00 |
| 8 | 1.55 | 93.01 | 4.28 | 0.00 |
| 24 | 3.64 | 83.35 | 6.38 | 0.05 |
| 48 | 4.43 | 88.51 | 3.97 | 0.08 |
| 72 | 8.68 | 79.24 | 6.43 | 0.37 |
| 120 | 10.28 | 78.25 | 5.25 | 0.61 |
| 168 | 23.69 | 61.95 | 5.45 | 1.99 |
| 288 | 27.87 | 54.82 | 6.33 | 4.76 |
| 360 | 38.75 | 38.23 | 7.21 | 10.43 |

Others indicate radioactivity not associated with any peaks.
In addition some radioactivity was recovered in the condensate (on top of glass dish) which did not exceed >0.90% TAR at any single time point and other volatiles at any given time point did not exceed >0.16% TAR.

Half-Life of $^{14}$C-Acifluorfen in the 102 and 21 ppm Series Aqueous Photolysis Experiments. Data Compiled from MRID # 44195002.

| Concentration | DT50 (hours) | DT50(Days) | $R^2$ |
|---|---|---|---|
| 102 ppm | 352.52 | 14.69 | 0.97 |
| 21ppm | 298.13 | 12.42 | 0.95 |

HPLC Analysis of 360 hour Photolysis Solution from the N-label By Ion Chromatography and further Analysis of Individual Peaks Isolated by Ion Chromatography by Using the 0.1% Ammonium Bicarbonate and 0.1% TFA HPLC System. Data from MRID # 44195002.

| Ion Pair Chromatography | | 0.1% Ammonium Bicarbonate HPLC Method | | | 0.1% TFA HPLC Method | | |
|---|---|---|---|---|---|---|---|
| Peak ID | % TAR | Peak ID | Retention Time (min) | % TAR | Peak ID | Retention Time (min) | % TAR |
| Peak 1 | 3.16 | Peak 1 | 8 - 10 | 3.07 | Peak 1 | 8 –11 | 2.80 |
| Peak 2 | 5.26 | Peak 2 | 8 - 10 | 3.93 | Peak 2a | 8 – 12 | 3.84 |
| | | Peak 2a | 12 – 14 | 1.10 | Peak 2b | 14 – 16 | 1.42 |
| Peak 3 | 3.71 | Peak 3 | 13 – 15 | 3.08 | Peak 3a | 9 – 13 | 1.46 |
| | | | | | Peak 3b | 16 – 19 | 1.99 |
| Peak 4 | 6.64 | Peak 4 | 14 – 16 | 6.13 | Peak 4a | 9 – 12 | 0.67 |
| | | | | | Peak 4b | 13 – 16 | 2.36 |
| | | | | | Peak 4c | 17 – 20 | 3.10 |
| Peak 5 | 6.14 | Peak 5 | 14 - 16 | 5.46 | Peak 5a | 8 – 11 | 1.24 |
| | | | | | Peak 5b | 16 – 21 | 4.83 |
| Peak 6 | 3.97 | Peak 6 | 14 – 16 | 3.87 | Peak 6a | 9 – 11 | 0.55 |
| | | | | | Peak 6b | 17 – 22 | 3.46 |
| Parent | 47.12 | Parent | 15 - 17 | 45.10 | Parent | 22 - 23 | 47.1 |

Distribution of Radioactivity in Various Fractions in the Aqueous Photolysis Experiments Conducted with $^{14}$C-Aciflurofen (N-Label) and $^{14}$C-Aciflurofen (F-Label) at 102 ppm Concentration for a Period of 360 Hours. Data Compiled from MRID # 44195002.

| Label | % TAR in | | | | | |
|---|---|---|---|---|---|---|
| | HPLC Analysis of Photolysis Solution | | | $CO_2$ | Condensate | Other Volatiles |
| | Polar | Parent | Others | | | |
| N Label | 23.93 | 63.0 | 4.13 | 3.28 | 0.29 | 0.09 |
| F- Label | 24.41 | 57.12 | 5.61 | 5.04 | 0.34 | 0.17 |

Quantitation of $^{14}$C-residues in the Photolysis Solutions of N and F labeled $^{14}$C-BAS 9048 H Using Ion Pair Chromatography. Data is from MRID # 44195002.

| Label | % TAR in | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Photolysis Solution | HPLC Analysis of Photolysis Solution | | | | | | | | |
| | | P1 | P2 | P3 | P4 | P5 | P6 | Total of P1 to P6 Peaks | Others | Parent |
| N-Label | 91.07 | 1.66 | 3.89 | 3.11 | 4.451 | 2.29 | 4.22 | 19.58 | 3.64 | 67.87 |
| F - Label | 87.14 | 1.77 | 3.56 | 2.14 | 3.65 | 3.33 | 4.07 | 18.52 | 10.09 | 58.52 |

Others include radioactivity not accounted for by any peaks

## Photodegradation on Soil (GDLN 161-3)

To examine photodegradation on soil, a loamy sand soil was treated with $^{14}$C-acifluorfen (N and F labels) at a concentration of approximately 3.5 to 3.8 ppm and subjected to photolysis at 25°C and at a light intensity of 1700 ? Einsteins/$m^2$/se. Control samples were similarly treated soil samples which were maintained in the dark at the same temperature. Treated soil samples were subjected to 0 to 15 days of continuous illumination (equivalent to 30 days of 12 hours of light and 12 hours of dark per day). Control samples were analyzed at the same sampling times as the irradiated samples.

In the N-label irradiated soil, the $^{14}C$-$CO_2$ was the major residue occurring at a maximum concentration of 7.72% TAR at 15 DAT whereas in the dark control soils, the $^{14}C$-$CO_2$ accounted for less than 0.2% TAR. The majority of radioactivity in extractable residues was associated with the parent peak. The amount of parent declined from 93.5% TAR in 0 DAT soil to 81% TAR in 15 DAT soil. There were two unknowns UK1 and UK2 and their concentration ranged from 1.1 to 2.7% TAR for all time periods. In the dark control soils, the majority of the extractable radioactivity was also associated with parent. The radioactivity ranged from 93.5 to 95.8% TAR in 0 to 15 DAT soils and unknowns UK1 and UK2 were found at a concentration of less than 3% TAR.

With the F-label, $^{14}C$-$CO_2$ was also the major residue in the irradiated soil occurring at a maximum concentration of 6.94% TAR at 15 DAT whereas in the dark control soils, the $^{14}C$-$CO_2$ accounted for less than 0.2% TAR. The acifluorfen concentration in the F-label accounted for 93.1% TAR at 0 DAT and declined to 82.4% TAR by 15 DAT. In the dark controls, acifluorfen accounted for 86.7% TAR at 15 DAT. There were two unknowns UK1 and UK2 and the concentrations of each were less than 2.3% TAR.

The mass balance for both irradiated and dark control samples in both N and F labels were between 87.8 to 109% TAR. The non-extractable residues did not exceed 7% TAR in any irradiated and dark control samples of N and F label treated soils. The half-life values for acifluorfen in the irradiated soil or dark controls were not determined since the concentration in irradiated and dark control soils remained at 81 and 95.2% TAR respectively at 15 DAT. The half-life under soil photolysis conditions was greater than 15 days.

The results obtained with $CF_3$ label for both the irradiated and dark controls were similar to those observed with the $NO_2$-label. The results from both the $NO_2$ and the $CF_3$ label studies indicate that acifluorfen is photolytically stable on soil and therefore, photolysis is not a major degradation pathway for the metabolism of this compound in the soil.

Reference:

Venkatesh, K. and Oakley, W. 1997. Photolysis of $^{14}$C-Acifluorfen ($NO_2$ and $CF_3$ Label) on Soil. BASF Registration Document No.: 97/5057. 5/08/1997 MRID # 44412901. Characterization of Soil Collected from BASF, FTS, NC Used in the Soil Photolysis Study. Data compiled from MRID #4412901.

| **Characterization** | |
|---|---|
| % Sand | 85 |
| % Silt | 8 |
| % Clay | 7 |
| Textural Class | Loamy Sand |
| Cation Exchange Capacity (meq/100g) | 5.3 |
| % Moisture at 1/3 Bar [Field capacity] | 8.2 |
| % Organic Matter | 1.0 |
| pH | 6.6 |

Distribution of Radioactivity in Extractable Residues, Marcs, Volatiles and $CO_2$ in Irradiated and Dark Control Samples Treated with $^{14}$C-Acifluorfen ($NO_2$-label). Data Compiled from MRID #4412901.

| DAT | % Total Applied Radioactivity in | | | | |
|---|---|---|---|---|---|
| | Extractables | Non-Extractables | Volatiles | $CO_2$ | Total |
| Irradiated Soil | | | | | |
| 0[2] | 100.70 | 0.91 | 0.00 | 0.00 | 100.60 |
| 3 | 97.12 | 3.20 | 0.00 | 2.15 | 102.47 |
| 7 | 89.56 | 4.54 | 0.01 | 5.49 | 99.60 |
| 10 | 99.30 | 3.67 | 0.04 | 5.95 | 108.96 |
| 15 | 89.98 | 3.53 | 0.02 | 7.72 | 101.44 |
| Dark Control | | | | | |
| 0[2] | 100.70 | 0.91 | 0.00 | 0.00 | 101.6 |
| 3 | 98.70 | 1.27 | 0.03 | 0.01 | 100.01 |
| 7 | 98.12 | 2.03 | 0.04 | 0.03 | 100.22 |
| 10 | 99.16 | 3.91 | 0.02 | 0.09 | 103.18 |
| 15 | 98.93 | 4.99 | 0.05 | 0.13 | 104.10 |

HPLC Quantitation of Extractable $^{14}$C-Residues from the Irradiated and Dark Control Samples Treated with $^{14}$C-Acifluorfen ($NO_2$-label). Data Compiled from MRID #4412901.

| DAT | % Total Applied Radioactivity in | | | | |
|---|---|---|---|---|---|
| | Available* | UK1 | UK2 | BAS 9048 H | Others** |
| Irradiated | | | | | |
| 0[2] | 100.70 | 1.47 | 2.65 | 93.50 | 3.08 |
| 3 | 97.12 | 1.13 | 2.22 | 84.68 | 9.09 |
| 7 | 89.56 | 1.84 | 2.40 | 79.03 | 6.30 |
| 10 | 99.30 | 1.36 | 2.41 | 93.16 | 2.38 |
| 15 | 89.98 | 1.81 | 2.33 | 80.95 | 4.89 |
| Dark Control | | | | | |
| 0[2] | 100.70 | 1.47 | 2.65 | 93.50 | 3.08 |
| 3 | 98.70 | 1.45 | 2.71 | 92.41 | 2.13 |
| 7 | 98.12 | 1.21 | 2.99 | 90.27 | 3.66 |
| 10 | 99.16 | 0.00 | 2.55 | 95.80 | 3.91 |
| 15 | 98.93 | 0.00 | 2.40 | 95.15 | 1.38 |

*Available indicates the total extractable residues
**Others indicate radioactivity not accounted for by any peaks.

Distribution of Radioactivity in Extractable Residues, Marcs, Volatiles and $CO_2$ in Irradiated and Dark Control Samples Treated with $^{14}C$-Acifluorfen ($CF_3$-label) . Data Compiled from MRID #4412901.

| DAT | % Total Applied Radioactivity in | | | | |
|---|---|---|---|---|---|
| | Extractables | Marc | Volatiles | $CO_2$ | Total |
| Irradiated Soil | | | | | |
| 0 | 95.80 | 0.27 | 0.00 | 0.00 | 96.07 |
| 7 | 91.07 | 6.73 | 0.02 | 3.99 | 95.08 |
| 15 | 84.97 | 5.24 | 0.03 | 6.94 | 90.94 |
| Dark Control | | | | | |
| 0 | 95.80 | 0.27 | 0.00 | 0.00 | 96.07 |
| 7 | 87.43 | 2.55 | 0.29 | 0.10 | 87.82 |
| 15 | 88.72 | 2.91 | 0.02 | 0.12 | 91.77 |

HPLC Quantitation of Extractable $^{14}C$-Residues from the Irradiated and Dark Control Samples Treated with $^{14}C$-Acifluorfen ($CF_3$-label). Data compiled from MRID #4412901.

| DAT | % Total Applied Radioactivity in | | | | |
|---|---|---|---|---|---|
| | Available* | UK1 | BAS 9048 H | UK2 | Others** |
| Irradiated | | | | | |
| 0 | 95.80 | 0.00 | 93.14 | 2.04 | 0.62 |
| 7 | 91.07 | 0.57 | 87.93 | 2.03 | 0.54 |
| 15 | 84.97 | 0.55 | 82.42 | 2.01 | 0.00 |
| Dark Control | | | | | |
| 0 | 95.80 | 0.00 | 93.14 | 2.04 | 0.62 |
| 7 | 87.43 | 0.00 | 84.23 | 2.27 | 0.44 |
| 15 | 88.72 | 0.00 | 86.72 | 2.00 | 0.00 |

*Available indicates the total extractable residues
**Others indicates radioactivity not accounted for by any peaks.

| | |
|---|---|
| Half life of Acifluorfen in the Soil Photolysis Study. Data Compiled from MRID #4412901 | >15 Days |

## Aerobic Soil Metabolism (GDLN 162-1)

In the original aerobic soil metabolism study submitted to the Agency (ASD No. 82/040), four soils were treated with both $CF_3$ and $NO_2$ labels at a concentration of 5 ppm and incubated at 23°C under aerobic conditions. The soil samples were collected at various intervals and selected samples were extracted and analyzed by TLC. The metabolites were identified by co-chromatography and mass spectrometry. The mass balance, parent concentration and half-lives determined in this study are given below.

Aerobic and anaerobic soil metabolism was also conducted with $^{14}C$-sodium acifluorfen labeled in the $NO_2$ ring using a Mississippi/New Jersey loam soil mixture. The soil was treated at an application rate of 1.0 ppm and incubated between 22 and 24 C. The soils from the aerobic portion of the study were sampled at 0, 1, 3, 7, 14 days; and 1, 2, 3, 4 and 6 months. After one month of aerobic aging, four treated samples were amended with 2 g of D-glucose and flooded with distilled water for the anaerobic portion of the study. The anaerobic soils were sampled at one and two months after flooding.

In the aerobic soil metabolism study (ASD 84/088), the extractable residues declined from 98% TAR at 0 DAT to 76% after 6 months. The non-extractable residues accounted for 2.5% TAR at 0 DAT to 24% after six months. Volatiles accounted for less than 1% TAR. The TLC analysis of extractable residues showed that parent was the major component and accounted for 90% TAR at 0 DAT; it declined to 43% after six months. The amino and the desnitro analogs were minor metabolites each accounting for 2.4 to 3.1% TAR at six months. TLC origin materials (polar metabolites) were at 5 – 7% and non polar-metabolites were at 3% TAR. The half-life of parent was estimated to be 170 days by the first order reaction in the loam soil mixture ( ASD 84/088). The half-life of acifluorfen was also estimated from an average of two ring-labeled treatments in the original study (ASD 82/040) for four soil types. The half-lives were estimated to be Georgia sandy loam, 111 days; Kansas clay loam, 200 days; New Jersey silt loam, 108 days; and Virginia sandy loam, 193 days.

References:

Wargo, J. P., Ku, C., Norris, F. 1982. Metabolism of Carbon-14 Labeled MC-10978 in Kansas, Virginia, Georgia and New Jersey Soils under Aerobic and Anaerobic Conditions Accession No. 07134, Rhone-Poulenc Report No. ASD 82/040. MRID # 00122760.

Gemma, A. A. and J. P. Wargo. 1984. Metabolism of $^{14}C$-MC-10978 (Tackle) in Soil under Aerobic and Anaerobic Conditions". ASD 84/088. MRID # 00143572.

Looper, G. 1990. Phase 3 summary of Rhone-Poulenc Report No. ASD 82/040 (Accession No. 071324) and Report No. ASD 84/088. MRID #. 00143572.

Characterization of Soils Used in the Aerobic Soil Metabolism Studies of $^{14}C$-Acifluorfen.

| | Report ASD 82/040 | | | | Report ASD 84/088 |
|---|---|---|---|---|---|
| USDA Textural Classification | Sandy Loam | Sandy Loam | Silt Loam | Clay Loam | Loam* |
| Origin | Georgia | Virginia | New Jersey | Kansas | |
| % Sand | 76.4 | 60.4 | 6.3 | 24.4 | 35.6 |
| % Silt | 15.6 | 27.0 | 66.8 | 47.2 | 49.6 |
| % Clay | 8.0 | 12.6 | 26.9 | 28.4 | 14.8 |
| pH | 6.2 | 5.0 | 6.1 | 6.5 | 5.4 |
| % Organic Matter | 0.8 | 1.1 | 2.7 | 1.9 | 1.4 |
| CEC (meq/100g) | 3.9 | 7.0 | 11.3 | 12.9 | 9.4 |

* Mixture of two silt loams (1:1) collected from Mississippi and New Jersey

The Distribution of Radioactivity in Extractable Residues, Non-extractable Residues and $CO_2$ at various Time Intervals in a New Jersey Soil. Data Compiled from Report ASD 82/040.

| DAT | % Applied Radioactivity | | | |
|---|---|---|---|---|
| | Extractable Residues | $CO_2$ | Non-Extractable Residues | Total |
| $CF_3$ Ring UL | | | | |
| 0 | 99.3 | <0.01 | 1.0 | 100.3 |
| 3 | 98.6 | 0.1 | 2.2 | 100.96 |
| 7 | 95.5 | 0.2 | 3.0 | 98.7 |
| 14 | 93.4 | 0.4 | 3.6 | 97.4 |
| 63 | 88.2 | 0.6 | 4.1 | 92.9 |
| 112 | 90.2 | 0.8 | 4.9 | 95.9 |
| 490 | 84.1 | 1.2 | 8.8 | 94.1 |
| | | | | |
| $NO_2$ Ring UL | | | | |
| 0 | 97.5 | <0.1 | 2.5 | 100.0 |
| 7 | 94.4 | <0.1 | 6.6 | 101.1 |
| 14 | 96.0 | <0.1 | 5.3 | 101.7 |
| 63 | 83.3 | 0.1 | 15.8 | 101.4 |
| 126 | 80.3 | 0.1 | 18.8 | 105.7 |
| 392 | 74.7 | 0.1 | 16.5 | 98.8 |

The Distribution of Radioactivity in Extractable Residues, Non-extractable Residues and $CO_2$ at Various Time Intervals in a Kansas Soil. Data Compiled from Report ASD 82/040.

| DAT | % Applied Radioactivity | | | |
|---|---|---|---|---|
| | Extractable Residues | $CO_2$ | Non-Extractable Residues | Total |
| $CF_3$ Ring UL | | | | |
| 0 | 99.3 | <0.01 | 0.7 | 100 |
| 4 | 90.8 | 0.2 | 7.2 | 98.2 |
| 7 | 99.6 | 0.2 | 2.2 | 102.0 |
| 14 | 97.3 | 0.6 | 2.8 | 100.3 |
| 42 | 93.9 | 0.6 | 5.9 | 100.4 |
| 91 | 97.3 | 0.6 | 2.3 | 100.2 |
| 490 | 93.9 | 0.8 | 2.3 | 96.9 |
| | | | | |
| $NO_2$ Ring UL | | | | |
| 0 | 99.7 | <0.1 | 0.2 | 99.9 |
| 7 | 99.7 | 0.1 | 0.4 | 100.2 |
| 14 | 102.5 | 0.2 | 0.6 | 103.3 |
| 63 | 99.5 | 0.2 | 3.7 | 103.4 |
| 126 | 93.9 | 0.6 | 4.1 | 98.6 |
| 392 | 93.8 | 0.7 | 5.2 | 99.7 |

The Distribution of Radioactivity in Extractable Residues, Non-extractable Residues and $CO_2$ at Various Time Intervals in a Virginia Soil. Data Compiled from Report ASD 82/040.

| DAT | % Applied Radioactivity | | | |
|---|---|---|---|---|
| | Extractable Residues | $CO_2$ | Non-Extractable Residues | Total |
| $CF_3$ Ring UL | | | | |
| 0 | 97.5 | <0.01 | 2.6 | 100.1 |
| 4 | 95.1 | <0.01 | 4.5 | 99.6 |
| 7 | 95.0 | <0.01 | 2.0 | 97.0 |
| 14 | 97.6 | <0.01 | 2.5 | 100.1 |
| 56 | 96.4 | 0.3 | 3.6 | 100.3 |
| 91 | 91.3 | 0.4 | 2.4 | 94.1 |
| 490 | 81.4 | 0.6 | 7.4 | 89.6 |
| | | | | |
| $NO_2$ Ring UL | | | | |
| 0 | 99.5 | <0.1 | 0.5 | 100 |
| 7 | 99.6 | 0.1 | 0.4 | 100.1 |
| 14 | 98.3 | 0.2 | 0.5 | 99.0 |
| 63 | 96.7 | 0.3 | 2.0 | 99.0 |
| 126 | 101.9 | 0.7 | 3.0 | 105.6 |
| 392 | 71.5 | 0.9 | 1.6 | 74.0 |

The Distribution of Radioactivity in Extractable Residues, Non-extractable Residues and $CO_2$ at Various Time Intervals in a Georgia Soil. Data Compiled from Report ASD 82/040.

| DAT | % Applied Radioactivity | | | |
|---|---|---|---|---|
| | Extractable Residues | $CO_2$ | Non-Extractable Residues | Total |
| $CF_3$ Ring UL | | | | |
| 0 | 99.7 | <0.01 | 0.3 | 100 |
| 4 | 102.4 | 0.01 | 0.8 | 103.3 |
| 7 | 103.5 | 0.3 | 0.6 | 104.4 |
| 14 | 98.5 | 0.5 | 3.4 | 102.4 |
| 63 | 94.7 | 0.8 | 1.1 | 96.4 |
| 112 | 94.9 | 1.5 | 2.1 | 97.8 |
| 490 | 77.8 | | 10.2 | 90.9 |
| | | | | |
| $NO_2$ Ring UL | | | | |
| 0 | 99.0 | <0.1 | 1.0 | 100 |
| 7 | 87.7 | 0.1 | 1.8 | 89.6 |
| 14 | 94.5 | 0.4 | 1.0 | 95.9 |
| 63 | 87.2 | 0.9 | 2.5 | 90.6 |
| 126 | 81.4 | 2.3 | 3.9 | 87.6 |
| 392 | 96.3 | 2.6 | 3.9 | 102.6 |

Acifluorfen Concentration in the Extractable Residues of Various Soils Incubated under Aerobic Conditions. Data Compiled from Report ASD 82/040.

| DAT and Label | % Of Applied Radioactivity | | | |
|---|---|---|---|---|
| $CF_3$ Ring | Georgia | Kansas | New Jersey | Virginia |
| 0 | 100 | 100 | 100 | 100 |
| 14 | 85.3 | 89.6 | 88.0 | 94.6 |
| 56 | -- | -- | -- | 83.5 |
| 63 | 72.8 | -- | 85.8 | -- |
| 91 | -- | 65.9 | -- | 75.6 |
| 112 | 40.6 | -- | 57.9 | -- |
| | | | | |
| $NO_2$ Ring | | | | |
| 0 | 100 | 100 | 100 | 100 |
| 14 | 87.3 | 97.2 | 79.9 | 92.0 |
| 63 | 70.6 | 84.0 | 46.3 | 75.8 |

--, indicates samples were either not taken or not analyzed.
Additionally 3.5% of the applied radioactivity was identified as the methyl ester of acifluorfen in the New Jersey soil at 63 DAT. The amino analog and the desnitro metabolite were found in trace quantities and their combined total was <1% of the applied radioactivity.

Half-life of Acifluorfen in Various soils Incubated Under Aerobic Conditions. Data compiled from Report ASD 82/040.

| Label | Half-life (days ) in Soil from | | | |
|---|---|---|---|---|
| | Georgia | Kansas | New Jersey | Virginia |
| $CF_3$ Ring | 94 | 153 | 160 | 226 |
| $NO_2$ Ring | 128 | 247 | 56 | 160 |
| Average | 111 ± 17 | 200 ± 47 | 108 ± 52 | 193 ± 33 |

The Total Material Balance of Acifluorfen Determined by Combustion and Extraction of Soil Incubated with $^{14}C$-Acifluorfen ($NO_2$ Label) under Aerobic Conditions at Various Time Intervals in the ASD 84/088 Study.

| DAT | % TAR as determined by Combustion of Soil | Extractable Residues | Non-Extractable Residues |
|---|---|---|---|
| 0 | 103 | 97.5 | 2.5 |
| 1 | 100 | 96.6 | 3.4 |
| 3 | 91.5 | 93.3 | 6.7 |
| 7 | 101 | 95.1 | 4.9 |
| 14 | 96 | 92.5 | 7.5 |
| 28 | 94 | 90.9 | 9.1 |
| 56 | 96 | 87.0 | 13.0 |
| 84 | 101 | 75.4 | 24.6 |
| 112 | -- | 81.1 | 18.9 |
| 175 | 83 | 76.3 | 23.7 |

-- not determined

The Parent and Metabolite Concentrations in Extractable Residues at Various Time Intervals in Soil Incubated with $^{14}C$- Acifluorfen ($NO_2$ Label) under Aerobic Conditions as Determined by Thin Layer Chromatography in the ASD 84/088 Study.

| DAT | BAS 9048 H (MC-10109) | Amino Metabolite (MC-14621) | Desnitro Metabolite (MC-10879) | Origin | Other Polars | Non-Polars* |
|---|---|---|---|---|---|---|
| 0 | 89.7 | 0.07 | 0.21 | 0.34 | 1.06 | 0.40 |
| 1 | 85.4 | 0.22 | 0.16 | 0.63 | 1.19 | 0.49 |
| 3 | 87.1 | 0.27 | 0.29 | 1.38 | 1.65 | 1.39 |
| 7 | 81.5 | 0.42 | 0.47 | 1.73 | 1.95 | 1.41 |
| 14 | 77.9 | 0.67 | 0.63 | 1.88 | 2.19 | 1.96 |
| 28 | 71.2 | 0.57 | 0.77 | 2.62 | 5.27 | 4.39 |
| 56 | 66.1 | 2.17 | 1.55 | 4.20 | 4.84 | 2.75 |
| 84 | 59.8 | 1.93 | 1.44 | 2.05 | 3.44 | 2.44 |
| 112 | 52.5 | 3.13 | 2.26 | 6.29 | 6.45 | 3.97 |
| 175 | 43.0 | 2.39 | 3.11 | 4.79 | 6.86 | 3.09 |

*Contains methyl ester of acifluorfen (MC-10108)

## Anaerobic Soil (GDLN 162-2) / Anaerobic Aquatic metabolism (GDLN 162-3)

An anaerobic soil metabolism study was conducted as a component of the aerobic soil metabolism study. $^{14}$C-sodium acifluorfen labeled in the $NO_2$ ring was applied to a loam soil (Report ASD 84/088). The loam soil was treated at an application rate of 1.0 ppm and incubated between 22°C and 24°C. After one month of aerobic aging, four treated samples were taken and amended with 2 g of D-glucose and flooded with distilled water for the anaerobic portion of the study. The anaerobic soils were sampled at one and two months after flooding.

In the anaerobic soil, the extractable residues declined from 79% at 30 DAT to 62% at 60 DAT. An additional 5.6% and 2.6% TAR was recovered in the flooded water at 30 and 60 DAT, respectively. The non-extractable residues accounted for 15% at 30 DAT and 35% TAR at 60 DAT. The metabolites in the extractable residues of anaerobic soil were the acetamide of the amine analog (9.8 and 12.1% TAR), parent (9.0 and 4.0% TAR), amino analog (7.3 and 5.7% TAR) and desnitro analog (6.6 and 7.8% TAR), at one and two months, respectively. The desnitro, amino and the acetamide in the anaerobic soil extracts was confirmed by either GLC or GC/MS analysis.

An anaerobic aquatic metabolism study was also conducted (MRID # 43155201). In this study, a clay soil obtained from Mississippi was flooded with well water obtained from a near-by site, since the soil was used as the test system. After establishing anaerobic conditions for 30 days the test system was treated with $^{14}$C-sodium acifluorfen labeled in the $CF_3$ ring and samples were analyzed for a period of 1 year. A second set up of similarly treated material was used to obtain samples at the shorter time intervals of 0, 1, 3, 7 and 10 days. Acifluorfen rapidly declined from 95.5% TAR at 0 DAT to 11.9% TAR (combined total from soil and water) at 10 DAT. The half-life of acifluorfen was estimated to be 2.75 days for the water/soil system under anaerobic conditions. The major metabolite was the amino analog of acifluorfen formed by rapid reduction of the nitro group which increased from 1.6% TAR at 0 DAT to 70.7% TAR at 25 DAT and then declined to 64.6% TAR at 375 DAT. Acetamide was a minor metabolite found at a maximum concentration of 3.18% TAR at 375 DAT. The volatiles, humic acids, fulvic acids and the humin fractions each accounted for 0.05, 12.0, 3.0 and 4.6% TAR, respectively at 375 DAT.

References:

Gemma, A. A. and J. P. Wargo. 1984. Metabolism of $^{14}$C-MC-10978 (Tackle) in Soil under Aerobic and Anaerobic Conditions". Accession No. 254534. MRID # 00143572.

Panek M. G. and C. E. Reese. 1994. Anaerobic Aquatic Metabolism of $^{14}$C-Sodium Acifluorfen. BASF Report No. M9326. BASF Reg. Doc. No. 92/5073. MRID # 43155201.

The Total Material Balance of Acifluorfen Determined by Extraction of Soil Incubated with $^{14}$C-BAS 9048 H ($NO_2$ Label) under Anaerobic Conditions at Various Time Intervals in the ASD 84/088 Study.

| DAT | % of Total Radioactivity in | | | |
|---|---|---|---|---|
| | Soil | Water | Total | Non-Extractable Residues |
| 1 month | 78.9 | 5.6 | 84.5 | 15.5 |
| 2 months | 62.1 | 2.6 | 64.7 | 35.3 |

*Additionally 5.6 and 2.6% of the total recovered radioactivity was in water which is not included in the total.

The Parent and Metabolite Concentrations in Extractable Residues at Various Time Intervals in Soil Incubated with $^{14}$C- Acifluorfen ($NO_2$ Label) under Anaerobic Conditions as Determined by Thin Layer Chromatography in the ASD 84/088 Study.

| Time | % Applied in | | | | | | |
|---|---|---|---|---|---|---|---|
| | Acifluorfen (MC-10109) | Acetamide Metabolite (MC-14621) | Amino Metabolite (MC-14621) | Desnitro Metabolite (MC-10879) | Origin | Other Polars | Non-Polars* |
| 1 month | 9.84 | 9.84 | 7.32 | 6.59 | 16.7 | 16.8 | 1.22 |
| 2 month | 12.09 | 12.09 | 5.73 | 7.77 | 14.24 | 14.24 | 2.99 |

*Contains methyl ester of acifluorfen (MC-10108)
The food water also analyzed by TLC and found that Acetamide, amino and desnitro analogs were also present each at <1% of total radioactivity.

Characterization of Soil and Water Used in the Anaerobic Aquatic Study Conducted with $^{14}$C-Acifluorfen. Data Compiled from MRID # 43155201.

| Soil | |
|---|---|
| Source | Leland, Mississippi |
| % Sand | 19.3 |
| % Silt | 34.0 |
| % Clay | 46.7 |
| USDA Textural Class | Clay |
| Bulk Density (gm/cc) | 1.13 |
| CEC (meq/100 g) | 28.6 |
| % Moisture at 1/3 bar | 37.4 |
| % Organic Matter | 2.3 |
| pH | 7.0 |
| | |
| Water | |
| Source | Irrigation well near the soil collection site |
| pH | 8.14 |
| Conductivity | 0.59 mhos |
| Calcium (mg/L) | 74 |
| Magnesium (mg/L) | 23.3 |
| Sodium (mg/L) | 9.4 |
| Hardness mg Equivalent $CaCO_3$/L (mg/L) | 282 |
| SAR (Sodium Adsorption Ratio) | 0.24 |

Distribution and Material Balance of $^{14}C$ residues at Various Time intervals in the $^{14}C$-Acifluorfen Anaerobic Aquatic Study. Data Compiled from MRID # 43155201.

| DAT | % Applied in | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Aqueous | Soil ext1 | Soil ext2 | Fulvic Acids | Humic Acids | Non-Extractables | NaOH Volatile | Total |
| Setup I | | | | | | | | |
| 0 | 77.71 | 21.44 | -- | -- | -- | 0.87 | -- | 100.01 |
| 4 | 71.27 | 20.73 | -- | -- | -- | 9.04 | 0.04 | 101.08 |
| 6 | 60.48 | 29.04 | -- | -- | -- | 8.26 | 0.03 | 97.80 |
| 11 | 49.70 | 36.30 | -- | -- | -- | 11.19 | 0.06 | 97.25 |
| 25 | 37.71 | 45.85 | 4.93 | 0.84 | 2.91 | 2.38 | 0.12 | 94.74 |
| 60 | 17.00 | 57.85 | 4.99 | 0.84 | 5.24 | 3.97 | 0.17 | 90.05 |
| 90 | 14.71 | 61.03 | 4.16 | 1.89 | 6.25 | 4.63 | 0.16 | 92.81 |
| 124 | 11.91 | 63.81 | 3.76 | 2.22 | 5.94 | 5.43 | 0.09 | 93.14 |
| 180 | 11.93 | 64.39 | 4.75 | 1.69 | 11.38 | 4.86 | 0.00 | 98.99 |
| 270 | 13.36 | 57.58 | 4.23 | 1.46 | 10.61 | 5.09 | 0.08 | 92.40 |
| 375 | 13.56 | 58.16 | 3.43 | 3.00 | 12.05 | 4.58 | 0.05 | 94.81 |
| Setup II | | | | | | | | |
| 0 | 95.62 | 3.86 | 0.23 | -- | -- | -- | -- | 99.70 |
| 1 | 86.99 | 11.14 | 1.18 | -- | -- | -- | -- | 99.31 |
| 3 | 74.05 | 20.98 | 3.25 | -- | -- | -- | -- | 98.27 |
| 7 | 59.04 | 33.10 | 1.87 | 0.26 | 1.82 | 1.00 | -- | 97.07 |
| 10 | 44.16 | 44.12 | 2.48 | 0.79 | 2.47 | 1.28 | -- | 95.29 |

--, indicates not determined or analyzed.

Acifluorfen Concentration in the Water and Soil Extract at Various Time Intervals in the Anaerobic Aquatic Study. Data Compiled from MRID #43155201.

| DAT | % Applied in | | |
|---|---|---|---|
| | Water | Soil Extract | Total |
| 0 | 92.60 | 2.95 | 95.55 |
| 1 | 77.11 | 5.52 | 82.63 |
| 3 | 49.20 | 4.97 | 54.17 |
| 7 | 24.92 | 4.78 | 29.70 |
| 10 | 9.98 | 1.92 | 11.90 |

| | |
|---|---|
| Half life of Acifluorfen Determined in Anaerobic Aquatic Study conducted with $^{14}C$-Acifluorfen. Data from Setup II. Data Compiled from MRID #43155201 | 2.75 Days |

Summary of Residue Concentrations in the Water and Soil at Various Time Intervals in the $^{14}$C-Acifluorfen Anaerobic Aquatic Study from setup II. Data Compiled from MRID #43155201.

| Sample and DAT | % Applied in | | | | | | |
|---|---|---|---|---|---|---|---|
| | Polars | Acifluorfen | Acetamide | Amino | Apolars | Extract2 | Total |
| 0 DAT Aqueous | 2.92 | 75.04 | 0.20 | 0.24 | 0.02 | | |
| 0 DAT Extract | | 17.38 | | 0.97 | 0.31 | | |
| **0 DAT** | **2.92** [a] | **92.42** | **0.20** | **1.21** | **0.33** | **n.d.** | **97.08** |
| 25 DAT Aqueous | 2.88 | 0.55 | 0.00 | 32.87 | 0.28 | | |
| 25 DAT Extract | 1.92 | 1.99 | 0.49 | 37.91 | 1.00 | | |
| **25 DAT** | **4.80** [a] | **2.54** | **0.49** | **70.78** | **1.28** | **5.27** [b] | **85.16** |
| 60 DAT Aqueous | 1.37 | 0.54 | 0.38 | 14.46 | 0.36 | | |
| 60 DAT Extract | 1.29 | 0.07 | 1.00 | 52.28 | 0.34 | | |
| **60 DAT** | **2.66** [a] | **0.61** | **1.38** | **66.74** | **0.70** | **4.99** [b] | **77.08** |
| 90 DAT Aqueous | 1.30 | 0.56 | 0.38 | 12.19 | 0.08 | | |
| 90 DAT Extract | 0.70 | 1.49 | 1.36 | 56.32 | 0.95 | | |
| **90 DAT** | **2.00** [a] | **2.05** | **1.74** | **68.51** | **1.03** | **4.16** [b] | **79.49** |
| 124 DAT Aqueous | 0.94 | 0.40 | 0.43 | 10.15 | 0.19 | | |
| 124 DAT Extract | 1.61 | 1.11 | 2.22 | 57.97 | 0.961 | | |
| 124 DAT Extract 2 | 0.39 | 0.20 | 0.18 | 2.91 | 0.05 | | |
| **124 DAT** | **2.94** | **1.71** | **2.83** | **71.03** | **1.15** | | **79.66** |
| 180 DAT Aqueous | 0.77 | 0.55 | 0.51 | 10.02 | 0.10 | | |
| 180 DAT Extract | 3.70 | 5.23 | 1.91 | 52.23 | 1.34 | | |
| 180 DAT Extract 2 | 0.68 | 0.23 | 0.20 | 3.43 | 0.21 | | |
| **180 DAT** | **5.15** | **6.01** | **2.62** | **65.68** | **1.65** | | **81.11** |
| 270 DAT Aqueous | 0.92 | 0.25 | 0.37 | 11.84 | | | |
| 270 DAT Extract | 2.54 | 0.90 | 1.90 | 52.55 | | | |
| 270 DAT Extract 2 | 0.74 | 0.16 | 0.18 | 3.13 | | | |
| **270 DAT** | **4.20** [a] | **1.31** | **2.45** | **67.52** | | | **75.48** |
| 375 DAT Aqueous | 1.03 | 0.27 | 0.47 | 11.79 | | | |
| 375 DAT Extract | 4.06 | 1.41 | 2.57 | 50.13 | | | |
| 375 DAT Extract 2 | 0.44 | 0.11 | 0.14 | 2.72 | | | |
| **375 DAT** | **5.53**[a] | **1.79** | **3.18** | **64.64** | | | **75.14** |

n.d., second sediment extraction not done
[a] Sum of two or three polar peaks
[b] Extract 2 not analyzed by HPLC

## Aerobic Aquatic Metabolism (GDLN 162-4)

In an aerobic aquatic metabolism study (MRID # 42330601), a clay soil was obtained from Mississippi. It was flooded with well water obtained from a near by site and immediately treated with $^{14}$C-sodium acifluorfen labeled in the $CF_3$ ring. It was incubated in dark at 25°C for 35 days. Acifluorfen was relatively stable decreasing from 98% of TAR at 0 DAT to 81.8% TAR at 35 DAT (combined total for soil and water system). The ratio of $^{14}$C-residues in the water and soil changed from approximately 9:1 immediately post-treatment to 5.5 at 35 days. At 35 DAT, non-extractable residues were 11.3% TAR; $^{14}CO_2$ was 4.39% TAR; and other volatiles were 0.12% TAR. There were 10 minor metabolites in the soil extracts at 35 DAT and their combined total did not exceed 3.7% TAR. The half-life of acifluorfen was estimated to be 114 days for the water/soil system under aerobic conditions.

Reference:

Panek G. 1992. Aerobic Aquatic Metabolism of $^{14}$C -Sodium Acifluorfen. BASF Reg Doc. No. 92/5066. MRID #42330601

Characterization of Soil and Water Used in the Aerobic Aquatic Study Conducted with $^{14}$C-Acifluorfen. Data Compiled from Data Compiled from MRID #42330601.

| Soil | |
|---|---|
| Source | Leland, Mississippi |
| % Sand | 28.5 |
| % Silt | 30 |
| % Clay | 41.5 |
| USDA Textural Class | Clay |
| Bulk Density (gm/cc) | 1.05 |
| CEC (meq/100 g) | 21.4 |
| % Moisture at 1/3 bar | 33 |
| % Organic Matter | 1.6 |
| pH | 6.9 |
| | |
| Water | |
| Source | Irrigation well near the soil collection site |
| pH | 7.87 |
| Conductivity | 0.57 mhos |
| Calcium (mg/L) | 71 |
| Magnesium (mg/L) | 21 |
| Hardness mg Equivalent $CaCO_3$/L (mg/L) | 265 |
| SAR (Sodium Adsorption Ratio) | 0.24 |

Distribution and Material Balance of $^{14}$C-Residues at Various Time Intervals in the Aerobic Aquatic study Conducted with $^{14}$C-Acifluorfen. Data Compiled from MRID #42330601.

| DAT | % Applied in | | | | |
|---|---|---|---|---|---|
| | Water | Sediment Extract | Non-extractables | Volatiles* | Total |
| 0 | 91.01 | 7.13 | 2.13 | 0.00 | 100.27 |
| 3 | 75.28 | 27.67 | 2.14 | 0.31 | 105.40 |
| 10 | 62.84 | 32.26 | 4.19 | 1.13 | 100.41 |
| 21 | 61.42 | 25.45 | 9.29 | 3.01 | 99.17 |
| 35 | 52.74 | 34.04 | 11.02 | 4.39 | 102.18 |

*Volatiles represent radioactivity in the base traps.

Acifluorfen Concentration in the Water and Sediment Extract at Various Time Intervals in the Aerobic Aquatic Study Conducted with $^{14}$C-Acifluorfen. Data Compiled from MRID #42330601.

| DAT | % Applied in | | |
|---|---|---|---|
| | Water | Sediment Extract | Total |
| 0 | 91.0 | 6.9 | 98.0 |
| 3 | 73.7 | 21.0 | 97.3 |
| 10 | 60.3 | 26.2 | 88.0 |
| 21 | 59.2 | 12.7 | 78.0 |
| 35 | 52.8 | 25.1 | 81.8 |

Bound Residue Fractionation of Non-Extractables at Various Time Intervals in the Aerobic Aquatic Study Conducted with $^{14}$C-Acifluorfen . Data Compiled from MRID #42330601.

| DAT | % Applied in | | | | |
|---|---|---|---|---|---|
| | Humic Acids | Fulvic Acids | Remaining aqueous | Residue | Total |
| 10 | 2.00 | | | 0.61 | 2.61 |
| 21 | 3.04 | 1.76 | 0.22 | 1.46 | 6.48 |
| 35 | 4.50 | 4.45 | 0.96 | 1.80 | 11.70 |
| | | | | | |

| | |
|---|---|
| Half life of Acifluorfen Determined in Aerobic Aquatic study. Data Compiled from MRID #42330601 | 114 Days |

## Leaching and Adsorption/Desorption (GDLN 163-1)

The trifluoromethyl labeled parent acifluorfen-free acid (5-(2-chloro-α, α, α-trifluoro-p-tolyloxy)-2-nitrobenzoic acid) had very low affinity for all the four soils used in the study. The Kads values were 0.148 for sand soil, 0.346 for sandy loam soil, 1.51 for the loam soil and 1.87 for the low organic (1.6%) clay soil and 3.1 for the high organic (3.2%) clay soil. The Koc values for sand, sandy loam, loam, low organic clay and high organic clay soils were 50.22, 73.52, 56.96 198.7 and 168.89, respectively.

Reference:

Suter, P. 1993. Adsorption and Desorption of Acifluorfen on Representative Agricultural Soils. BASF Report No. M9312. BASF Reg. Doc. No. 93/5042. MRID # 42793501

## Leaching and Adsorption/Desorption of Degradates (GDLN 163-1)

An adsorption/desorption study was conducted with $^{14}$C-acifluorfen amine (the only major metabolite found at >10% TAR in environmental fate studies - 76.8% in anaerobic aquatic at 10 DAT [MRID # 43155201]) on four soils. The Kads values for the sand, clay, loam and loamy sand were 1.25, 12.11, 19.34 and 47.01% TAR, respectively. The Koc values were 431 for sand, 652 for clay, 741 for loam and 7368 for the loamy sand indicating that amine acifluorfen is immobile in loamy sand, of low mobility in loam and clay soil, and of medium mobility in sand soil.

Reference:

Mills, C. and A. G. Goetz. 1997. Adsorption/Desorption of $^{14}$C-BH 9048-A (Amino Acifluorfen) On Soil. BASF Reg. Doc. No. 97/5334. MRID No. 44412902.

Characterization of Soils used to Assess the Adsorption/Desorption of $^{14}$C-Acifluorfen. Data Compiled from MRID # 42793501.

| USDA Textural Classification | Sand | Sandy Loam | Loam | Clay | Clay |
|---|---|---|---|---|---|
| Origin | North Carolina | California | Illinois | Mississippi | North Dakota |
| % Sand | 96 | 63 | 29 | 28.5 | 28 |
| % Silt | 2 | 24 | 48 | 30 | 23 |
| % Clay | 2 | 13 | 23 | 41.5 | 49 |
| pH | 6.9 | 7.5 | 6.3 | 6.9 | 6.8 |
| % Organic Matter | 0.5 | 0.80 | 4.5 | 1.6 | 3.2 |
| Bulk Density (g/cc) | 1.57 | 1.32 | 1.09 | 1.05 | 1.1 |
| CEC (meq/100g) | 1.9 | 12.5 | 22.4 | 21.4 | 41.0 |

Adsorption/Desorption Constants for $^{14}$C- Acifluorfen. Data Compiled from MRID # 42793501.

| | | Soil Type | | | | |
|---|---|---|---|---|---|---|
| | | Sand | Sandy Loam | Loam | Clay | Clay |
| Adsorption | $K_{ads}$ | 0.15 | 0.35 | 1.51 | 1.87 | 3.10 |
| | $K_{oc}$ | 50.22 | 73.52 | 56.96 | 198.70 | 164.89 |
| | l/n | 0.82 | 0.89 | 0.85 | 0.82 | 0.75 |
| | | | | | | |
| | | | | | | |
| Desorption | $K_{des}$ | 0.46 | 0.65 | 3.06 | 2.95 | 4.47 |
| | $K_{oc}$ | 156.88 | 137.93 | 115.57 | 313.34 | 237.37 |
| | l/n | 0.89 | 0.83 | 0.84 | 0.81 | 0.77 |

Characterization of Soils used to Assess the Adsorption/Desorption of $^{14}C$-Amine Acifluorfen. Data Compiled from MRID # 44412902.

| USDA Textural Classification | Sand | Clay | Loam | Loamy Sand |
|---|---|---|---|---|
| % Sand | 96 | 28 | 29 | 80 |
| % Silt | 2 | 23 | 48 | 14 |
| % Clay | 2 | 49 | 23 | 6 |
| pH | 6.9 | 6.8 | 6.3 | 5.7 |
| % Organic Matter | 0.5 | 3.2 | 4.5 | 1.1 |
| Bulk Density (g/cc) | 1.57 | 1.10 | 1.09 | 1.38 |
| CEC (meq/100g) | 1.9 | 41.0 | 22.4 | 4.9 |

Adsorption/Desorption Constants for $^{14}C$- Amine Acifluorfen. DataCompiled from MRID # 44412902.

| | | Soil Type | | | |
|---|---|---|---|---|---|
| | | Sand | Clay | Loam | Loamy Sand |
| Adsorption | % TAR Sorbed | 28.7 | 79.5 | 85.5 | 92.7 |
| | $K_{ads}$ | 1.25 | 12.11 | 19.34 | 47.01 |
| | $K_{oc}$ | 431 | 652 | 741 | 7368 |
| | L/n | 0.802 | 0.869 | 0.893 | 0.936 |
| | $R^2$ | 0.951 | 0.999 | 0.999 | 0.988 |
| | | | | | |
| Desorption | % TAR Desorbed | 55.9 | 4.4 | 11.1 | 11.8 |
| | $K_{des}$ | 1.53 | 0.04 | 0.11 | 0.14 |
| | L/n | 1.075 | 1.105 | 0.890 | 1.177 |
| | $R^2$ | 0.838 | 0.776 | 0.926 | 0.953 |

# APPENDIX 4: RESPONSE TO EPA "INTEGRATED ENVIRONMENTAL RISK CHARACTERIZATION" CHAPTER

## APPENDIX 4: RESPONSE TO EPA "INTEGRATED ENVIRONMENTAL RISK CHARACTERIZATION" CHAPTER

In its analysis of the potential of sodium acifluorfen to present a risk to contaminate drinking water supplies, EPA uses results from a number of studies as evidence for its assumptions. BASF has evaluated the values that EPA has used in its SCI-GROW calculations and is rebutting those values in the arguments presented below.

**Potential for acifluorfen to contaminate ground water**: In its discussions on the potential for acifluorfen to contaminate ground water, EPA relies heavily on the results obtained in a prospective groundwater study performed in 1988 which BASF believes has serious deficiencies. That study was performed using techniques no longer considered appropriate for a PGW. BASF believes the results of that study should be discounted and that the Agency should rather rely on results obtained from the five prospective/retrospective study sites and a new PGW study which was performed recently and which has been cited by the Agency in its reviews. In addition, results from the National Water Quality Assessment Program (NAWQA), which examined 2,604 samples from 894 wells, showed only one unverified detect of acifluorfen, giving further evidence that acifluorfen is not a significant ground water contaminant.

**Half-life calculations:** In a study carried out by Gaston et al. (cited below), the half-life of acifluorfen obtained in the laboratory using soil columns instead of bioflasks was in excellent agreement with results obtained in field studies performed by BASF with the compound. BASF believes that the results from these modified laboratory studies are more appropriate for the SCI-GROW calculations.

**Sorptive properties of acifluorfen and amino acifluorfen: :** Data presented by Gennari et al. and by Locke et al. (cited below) have shown that binding of acifluorfen to soils is dependent on organic carbon content and pH. The behavior of the compound in soil, as described by these investigators, would contradict the KOC values chosen by EPA to use in its SCI-GROW calculations. In addition, the same authors have shown that the primary transformation product of acifluorfen, amino acifluorfen, is highly sorbed to the soil as well.

Presented below are the data which support these conclusions:

## 1988 Small Scale Prospective Groundwater Study

At the Agency's request, a prospective groundwater study (PGW) was initiated in 1988 to investigate the leaching potential of sodium acifluorfen in Wisconsin (MRID 41172801). The PGW site was located at the University of Wisconsin agricultural research station in Hancock at a site with a highly vulnerable sandy soil. The field was divided into four sections or quadrants where well and lysimeter stations were installed. A single application of sodium acifluorfen was made to the 4.5 acre field at a rate of 0.75 lb ai/ac, twice the label rate allowed on the label. However, the behavior of the compound in this study was not consistent with behavior expected in a PGW.

In the final study report the author, in an attempt to explain study result anomalies, wrote "*Contamination problems due to lack of flushing of the lysimeters between samples may have also presented artifacts that make interpretation of the results difficult*" (final report page 19). The author continued, "*Also there was no increase or decrease in the magnitude of the residue with time as would be expected of a well defined plume movement through the soil . These observations suggest that residue detects may not be the result of solute flow through the soil but could be the result of preferential flow either through channeling in the soil or along the casing of the lysimeter*." (final report pages 19 and 20).

In an effort to explain the anomalous behavior of the compound in this study, literature papers were submitted at the time of submission which attributed the discontinuity to funnel flow. Fortunately, a much larger body of knowledge regarding preferential flow and funnel flow has been developed since 1988 when this study was conducted. However, even with a larger body of knowledge, the data generated in this study are difficult to explain. As an example, well cluster 16 consisted of three wells with screening depths of 5.5 m, 6.72 m and 8.27 m. The water table depth in the three wells was the same depth (5.2 m). On 10/27/88 the 5.5 m well had no detectable residues of acifluorfen measured. However on the same date the 6.72 m well had 11 µg/L acifluorfen detected. Nine days later both wells were sample again but this time the shallow well had 8 µg/L acifluorfen detected and the deeper well had no detectable residues of acifluorfen. Cluster 16 later in time produced the highest residues in the study.

We believe the poor field techniques used to set this site up are responsible for the confusing data generated in this study. Therefore it is instructive to review the techniques used to install wells at this field site. All wells were hand augured. The well casing material was PVC and was beat into the annulus with a mallet. The wells were not cased at the surface with concrete. Based on most state laws today, these installation techniques could not be used. Installation techniques such as those used in this 1988 study have been prohibited in efforts to protect groundwater quality. While it could be argued that the field techniques used at the Hancock can work, the results obtained using

them in this study strongly argue against this assertion. Additionally, tracer information would have been a valuable asset in this study but none was applied.

Results from this study lack scientific or regulatory credibility. BASF would suggest that the most useful scientific information that can be obtained from this study is that poor field technique creates useless results. Furthermore, once we consider the five retrospective groundwater sites conducted in 1989-1990 (and the results of the PGW recently conducted by Valent Corporation) it becomes difficult to argue that the Wisconsin study has any value at all.

## Retrospective Ground Water Study 1989-1990

In 1989 a small scale retrospective study was initiated with acifluorfen (MRID 42152201) in an attempt to answer the leaching vulnerability questions left unanswered by the 1988 Hancock study. Five sites were included in this additional study. Each site was selected for its representation of a specific growing region (based on soybean, peanut or rice use patterns). Additionally, prior acifluorfen use history as well as groundwater vulnerability were also considered when selecting sites. The first two criteria specified (crop distribution and prior use) are straightforward to evaluate. Actual site vulnerability however can be subjective. To eliminate site selection subjectivity, DRASTIC scores were used to maximize the probability of selecting vulnerable sites. Final site selection was made from an examination of soils, hydrogeology and annual precipitation information. Site characterization and depth to ground water information are summarized in Table 1.

> Since soil texture, organic matter content, depth to ground water and annual precipitation are used as selection criteria, it is unquestionable that the sites chosen were extremely vulnerable. Soil particle size distributions indicated all sites were situated on soils with 82% sand composition (or greater) below a soil depth of 24 inches. Depth to ground water varied from a minimum of 0.4 feet to a maximum of 15.6 feet. Three clusters of three wells were installed at each study location. A summary of total rainfall and compound applications during the study period are presented in Table 2.

By study completion, all sites had received at least three years of sequential acifluorfen applications. Measured rainfall quantities during the conduct of the studies were close to or exceeded historical rainfall amounts. Well water samples were collected monthly for 12 months following the last test substance application. The analytical method used for measuring samples had a LOQ of 1 ug/L. By study completion not a single sample collected during the duration of the study had quantifiable residues of acifluorfen.

Results from this study indicates that acifluorfen is not a groundwater concern under conditions of use even on highly vulnerable soil.

**Table 1. Summary of site soil characteristics and depth to ground water.**

| Site | Depth | Sand | Silt | Clay | Texture | OM% | pH | Depth to GW |
|---|---|---|---|---|---|---|---|---|
| VA | 0-1 | 70 | 20 | 10 | sl | 1.8 | 5.1 | 3.7-10.5' |
| | 1-2 | 68 | 16 | 16 | sl | 0.5 | 5 | |
| | 2-3 | 82 | 8 | 10 | ls | 0.1 | 4.9 | |
| | 3-4 | 96 | 2 | 2 | s | 0.1 | 5 | |
| | 4-5 | 96 | 2 | 2 | s | 0.2 | 5 | |
| | 5-6 | 96 | 2 | 2 | s | 0.1 | 5.1 | |
| TN | 0-1 | 44 | 38 | 18 | l | 1.3 | 7.6 | 8.1-15.6' |
| | 1-2 | 44 | 44 | 12 | l | 0.7 | 7.9 | |
| | 2-4 | 90 | 6 | 4 | s | 0.1 | 8.1 | |
| | 4-6 | 94 | 2 | 4 | s | 0.1 | 8 | |
| | 6-8 | 94 | 2 | 4 | s | 0.4 | 7.8 | |
| | 8-9 | 94 | 2 | 4 | s | 0.5 | 8.1 | |
| NC | 0-1 | 85 | 6 | 9 | ls | 2.3 | 5.3 | 0.4-4.7' |
| | 1-2 | 83 | 4 | 13 | ls | 0.3 | 4.7 | |
| | 2-3 | 83 | 4 | 13 | ls | 0.1 | 4.6 | |
| | 3-4 | 91 | 4 | 5 | s | 0.1 | 4.7 | |
| | 4-5 | 95 | 2 | 7 | s | 0.1 | 4.6 | |
| | 5-6 | 95 | 0 | 5 | s | 0.1 | 4.8 | |
| | 6-7 | 96 | 1 | 5 | s | 0.1 | 4.9 | |
| IN | 0-1 | 60 | 25 | 15 | sl | 2.3 | 5.1 | 4.1-10.9' |
| | 1-2 | 66 | 17 | 17 | sl | 1.2 | 5.6 | |
| | 2-4 | 82 | 11 | 7 | sl | 0.1 | 5.8 | |
| | 4-6 | 92 | 5 | 3 | s | 0.1 | 6 | |
| | 6-8 | 92 | 5 | 3 | s | 0.1 | 7.9 | |
| ND | 0-1 | 77 | 14 | 9 | sl | 2.7 | 7.8 | 1.6-6.4' |
| | 1-2 | 85 | 8 | 7 | ls | 0.5 | 8.7 | |
| | 2-4 | 87 | 4 | 9 | ls | 0.1 | 9.3 | |
| | 4-6 | 93 | 2 | 5 | s | 0.1 | 8.9 | |

**Table 2. Summary of site precipitation and acifluorfen applications.**

| Site | Yr. | Precipitation (in) | | App. Date | Rate (lb ai/a) | Seq. Apps. Prior to study |
|---|---|---|---|---|---|---|
| | | Historical | Actual | | | |
| VA | '89 | 42.1 | 51.4 | 7/6/89 | 0.26 | 2 yrs |
| TN | '89 | 50.5 | 61.6 | 5/31/89 | 0.23 | 3 yrs |
| NC | '89 | 50.8 | 42.8 | 6/27/89 | 0.23 | 4 yrs |
| IN | '89 | 45.0 | 53.9 | 6/30/89 | 0.21 | 1 yr |
| | '90 | 45.0 | 43.4 | 7/10/90 | 0.25 | |
| ND | '89 | 19.5 | 22.1 | 6/30/89 | 0.43 | 1yr |
| | '90 | 19.5 | 13.6 | 6/23/90 | 0.25 | |

## 1999 Small Scale Prospective Groundwater Study

In its **"Response to Valent's 30-day Error Correction Comments on the Lactofen Drinking Water Assessment"** dated October 2, 2000, the Agency reports " Valent is currently conducting a small-scale prospective ground-water study in St. Joseph County Michigan. The registrant has just submitted the third interim reports (MRID 4521490001). The bromide tracer has reached ground water in 7 of 9 of the shallow (15 feet below the surface) monitoring wells and 2 of the deep (25 feet below the surface) monitoring wells. Acifluorfen has been detected in some soil pore water collected in the shallow suction lysimeters, but to date has not been found in or below any of samples collected at or below the 9-foot deep the suction lysimeters."

Since the Agency has accepted a protocol from Valent Corporation to conduct the study in Michigan, essentially repeating the conditions of the 1988 study performed by BASF, we assume that the Agency believes that the acifluorfen PGW was flawed. According to the Agency's summary no acifluorfen has reached ground water, which is consistent with all the data available except the acifluorfen PGW. Further, monitoring result discussed below also demonstrate that acifluorfen is not a significant ground water contaminant.

## Results from National Water Quality Assessment Program (NAWQA)

Acifluorfen is included in the residue methods used by NAWQA. The NAWQA acifluorfen analytical method was developed for an LOQ of 0.035 ug/L in groundwater. Results from the NAWQA program to date indicate that although 2,604 samples have been collected from 894 wells, only one sample with detectable residues of acifluorfen (0.19 ug/L) has been identified.

It is important to remember that before water is distributed for public consumption it is commonly treated with a chlorine source, charcoal, or a combination of treatments. These common treatments can significantly reduce concentrations of organic chemicals.

This means any detectable residue in the NAWQA program would potentially be lower by the time it was distributed for consumption as drinking. We believe the NAWQA data is very consistent with all of the experimental work conducted on the compound. The source of this data may be found at the USGS NAWQA web site (http://wwwdwatcm.wr.usgs.gov/ccpt/pns_data/data.html).

Results from monitoring to date indicate one unverified detect (0.19 ug/L) based on 2,604 measured samples. Again results from this study indicates that acifluorfen is not a groundwater concern under conditions of use.

## The Effect of Soil Properties on Sorption of Acifluorfen

In Appendix J "Drinking water exposure assessment for lactofen, lactofen derived acifluorfen, and acifluorfen-sodium," and in Appendix K from EPA's preliminary risk assessment for sodium acifluorfen, some assumptions are used to justify KOC selection as input for the SCI-GROW model.

The assumptions used by the Agency regarding the behavior of the molecule with change in pH are not correct. The acifluorfen molecule has a carboxylic acid group which gives the compound a pKa of 3.5 (Roy et al., 1983). Therefore acifluorfen should be fully dissociated in agricultural soils, since pH's are managed well above this pKa (Gennari et al., 1994).

The sorptive properties of acifluorfen were examined in a paper by Gennari et al. on five European soils. In this study two soils were entisols, one was an inceptisol, one was an andisol and one was a istosol (not NRCS taxonomy). Measured acifluorfen KOC's in this paper ranged from 65.3 to 560.6. The conclusions reached by Gennari et al. regarding acifluorfen binding in soil was that it was dependent on organic carbon content and soil pH. Destruction of the organic carbon portion of the soils with $H_2O_2$ resulted in lost sorption by the soils. The pH effect was attributed to the net increase in positive charge on the surfaces of iron oxides in soil (not pKa). Since a change in binding was due to the increased charge on the clay portion of the soil, as pH decreased acifluorfen adsorption increased. This effect is the opposite of that presented by EFED in their SCI-GROW calculations.

A second paper by Locke et al. (1997) also examined the binding properties of acifluorfen with twelve US soils. However Locke et al. attributed sorption to organic carbon content, cation exchange capacity (CEC) and soil acidity. The physical properties of the soil investigated by Locke et al. are presented in Table 5.

**Table 5. Soil physical properties from page 287 Table 1 after Locke, Gaston and Zablotowicz (1997).**

| Soil | pH (1:1 $CaCl_2$) | Clay Content % | CEC (cmol/kg) | OC (g/kg) | Kd L/kg | KOC L/kg |
|---|---|---|---|---|---|---|
| Dundee Loam (0-10 cm) | 5.59 | 13.1 | 12.1 | 7.47 | 0.48 | 64.3 |
| Dundee Silt Loam (0-10 cm) | 5.08 | 35.2 | 19.5 | 7.48 | 0.56 | 74.9 |
| Dundee Silt Loam (0-5 cm) CT | 5.29 | 22.0 | 14.3 | 11.9 | 1.30 | 109.5 |
| Dundee Silt Loam (0-5 cm) NT | 5.13 | 22.0 | 16.7 | 22.4 | 3.15 | 140.5 |
| Lafitte muck (0-10 cm) | 4.10 | 20.0 | 78.0 | 191.3 | 89.6 | 468.4 |
| Mahan loamy fine sand (0-13 cm) | 4.20 | 4.7 | 3.8 | 12.0 | 1.66 | 138.0 |
| Mahan loamy fine sand (26-36 cm) | 4.40 | 14.8 | 4.1 | 0.10 | 0.86 | 8600 |
| Miami silt loam CT (0-10 cm) | 6.16 | 40.0 | 14.1 | 19.0 | 1.10 | 57.9 |
| Miami silt loam NT (0-10 cm) | 6.36 | 40.0 | 15.6 | 35.0 | 1.80 | 51.4 |
| Sharkey clay (0-10 cm) | 6.00 | 61.0 | 43.7 | 16.9 | 2.16 | 128.2 |
| Ships clay (0-10 cm) | 7.50 | 50.0 | 40.1 | 8.34 | 0.61 | 73.1 |
| Weswood silt loam (0-10 cm) | 7.70 | 21.0 | 16.4 | 3.11 | 0.30 | 95.2 |

Although Locke et al. attributed increased acifluorfen sorption to organic carbon content, cation exchange capacity (CEC) and soil acidity, our examination of their data indicate that the overwhelming contributor to sorption was made by the soil organic carbon content. In Figure 1, a correlation of soil physical properties with Kd are presented. The only property with a significant correlation to sorption was % OC (r=0.68).

The following relationship was derived by Locke et al. (1997) to describe sorption:

$$Kd = -1.05 + (0.102(OC))^{**} + 12769(CEC)[H]^{**} \quad R^2 = 0.99$$

*Where OC is organic carbon content, CEC cation exchange capacity and H soil pH*

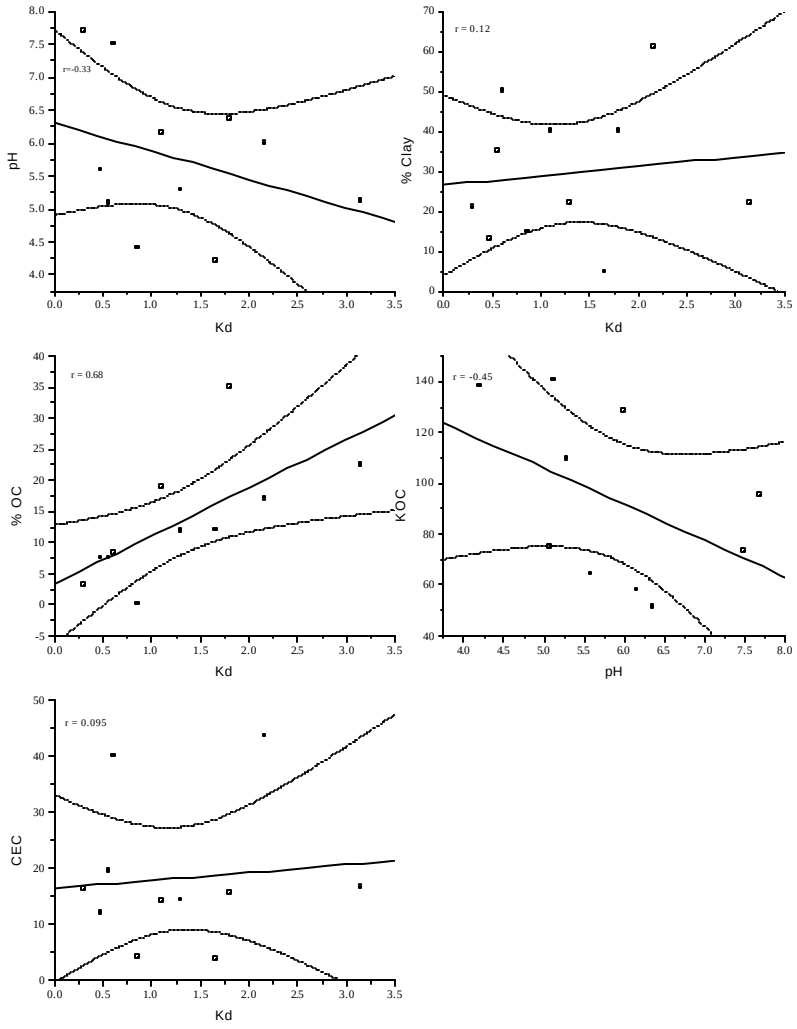



**Figure 1. Correlation matrix of Kd vs other physical properties in Table 5. The only relationship with significance is the relationship between Kd and %OC content. Lafitte muck was excluded since it is not a mineral soil.**

BASF was able to confirm the authors' relationship using multiple regression (R=0.95). The arithmetic mean of KOC's obtained by Locke (without the 8600 KOC soil) was 127.4 (ml/g). This value is higher than those used in surface and ground water modeling by EFED.

Results based on the literature cited (and registrant data) indicate no scientific validity to the approach used to select a KOC value for acifluorfen by the Agency for its SCI-GROW calculations.

*Sorption and amino acifluorfen*

The primary transformation product of acifluorfen is amino acifluorfen. Andreoni et al., (1994) found that acifluorfen degraded to amino acifluorfen under both oxygen unlimited and oxygen limited conditions. Locke et al. (1997) observed transformation of acifluorfen to amino acifluorfen within 96 hours at 9.9% and 17.8% on Dundee and Sharkey soils respectively. The authors also looked at the effect of soil temperature on the degradation of acifluorfen indirectly through a binding experiment. They stated that decreased binding was observed in the 4°C treatment compared to the 25C ° treatment. They also stated that the decrease in binding indicated that the transformation of acifluorfen to amino acifluorfen was microbially mediated. In addition, they stated that amino acifluorfen had a high affinity for soil, which explained the high sorption irreversibility they observed ($Kd_f$ 41.3 and 47.2 for Dundee and Sharkey soils respectively). In an additional paper by Gaston et al. (2000), it was determined that intermediate products of acifluorfen were apparently highly sorbed as well.

From the literature it is clear that once degradation of acifluorfen occurs, transformation products adsorb strongly to soil.

## Laboratory Estimation Of Field Half-Life

The regulatory community has often questioned whether laboratory data accurately describe the true half-life of a compound in the environment. Industry has maintained that aerobic soil metabolism studies as conducted according to the current guidelines are not suitable for predicting compound dissipation rates in the field.

As a component of the study conducted by Gaston L.A. et al. (2000), a comparison of degradation rates for acifluorfen between a typical aerobic soil metabolism study design and a study using soil cores (columns) in the laboratory was made. A summary of the results obtained from this comparison are presented in Table 6.

**Table 6. Summary of Table 5 data from L. A. Gaston et. al. (2000), page 117**

| Soil | Depth (cm) | Aerobic App. Batch | Cores | | | |
|---|---|---|---|---|---|---|
| | | | CT 1 | CT 2 | NT 1 | NT 2 |
| | | ----- Half-life (Days) ----- | | | | |
| CT | 0-10 | 74 d | 15.4 d | 12.8 d | -- | -- |
| | 20-30 | 169 d | 12 d | 43.3 d | -- | -- |
| NT | 0-10 | 108 d | -- | -- | 11.7 d | 7.2 d |
| | 20-30 | 165 d | -- | -- | 63 d | 347 d |

CT = conventional till, NT = non-till

The results for acifluorfen obtained by Gaston et al. (2000) using a laboratory system similar to those typically used in guideline aerobic studies are in good agreement with results obtained by BASF (MRID 00143572). The results obtained from the core (column) portion of the Gaston et al. (2000) study are in good agreement with results obtained from field studies by BASF and others (Table 7).

In their study Gaston et al. explained that half-live values calculated from biometer flasks results may have been artificially long with respect to soil cores (columns) at similar water content. The difference observed between the bioflask results and the soil core results (column) were attributed to poorer aeration in the cores (particularly below the surface) compared to the bioflasks. Bioflasks have a very unnatural surface to volume ratio compared to actual field conditions. In the abstract of Gaston et al. (2000), the authors state *"Furthermore, first-order rate constants obtained from the batch study under-estimated acifluorfen degradation during transport. Faster acifluorfen degradation in the soil columns may have been due to poorer aeration compared to the batch systems."*

We are not convinced that aeration was the issue in this study but do believe that batch studies are highly artificial. The half-life values obtained by Gaston using bioflasks ranged from 74-169 days. The half-life values obtained by Gaston using soil columns ranged from 7.2 days to 63 days (less one sample at 347 days, for which the author had no explanation [personal communication]).

The degradation data for acifluorfen are consistent. The half-life of acifluorfen under realistic field conditions is relatively short. As previously stated, we believe the best estimate of a compound's true half-life in the field comes from field results. However, results from experiments such as those presented in Table 6 add considerable weight to a discussion that seems fairly conclusive - that aerobic laboratory study conditions are not suitable to estimate dissipation time in the field.

Results from this work indicate that the true field half-life of acifluorfen should range from 7.2 days to 63 days based on modified laboratory study results.

## Dissipation Time Of Acifluorfen Under Field Conditions

The half-life of acifluorfen under irrigated field conditions ranged from about 7.6 days to a maximum of 41 days based on data from the one failed 1988 PGW site and the five retrospective study sites. Table 7 summarizes the half-life values measured for the various field studies conducted with acifluorfen to date.

**Table 7 Summary of field half-life/ dissipation times for acifluorfen**

| Site | Study | DT50[(1)] / t 1/2[(2)] |
|---|---|---|
| IN - '90 | Retro. | 30.6 d [(1)] |
| ND - '90 | Retro. | 41.0[(1)] |
| IN - '89 | Retro. | 7.6 [(2)] |
| ND - '89 | Retro. | 13.9[(2)] |
| NC - '89 | Retro. | 14.8[(2)] |
| TN - '89 | Retro. | 21.8[(2)] |
| VA - '89 | Retro. | 14.1[(2)] |
| WI - '88 | PGW | 14.7 |
| | | |

(1) denotes data were recalculated using the Gustafson-Holden
(2) denotes data were calculated by simple regression.

Results from field work conducted to date indicate the half-life obtained for acifluorfen at different sites from different studies over various years are consistent. The average half-life of acifluorfen in the field is about 20 days.

## DRINKING WATER EXPOSURE ASSESSMENT

When asking why residues of acifluorfen are not detected in groundwater, the answer is to be found in an accurate appraisal of the compound's half-life in the field and its true binding characteristics as presented in the previous sections. It has been shown that once degradation occurs, transformation products of acifluorfen bind tightly to the soil. The reason that acifluorfen is not found in groundwater is due to the fact that it has a relatively short half-life and that its degradates bind strongly to soil.

The Agency has expended considerable effort to justify reducing experimentally determined KOC values in their SCI-GROW calculations for sodium acifluorfen in order to match results from the deficient PGW study. However the data presented by BASF in this report demonstrate that there is no scientific justification for the selection of the KOC value which the Agency has used in its SCI-GROW calculations. The Agency has hypothesized that acifluorfen KOC values will decrease with decreasing pH, presumably

by increasing the amount of the compound that is ionized. As described in the literature cited in this document, the molecule will be fully ionized in agricultural soils, so there can be no increase in ionization. In fact, the only pH effect observed in soil systems was the tendency for soil sorption to increase with lower soil pH (which increases the subsequent binding of acifluorfen).

Based on the experimentally determined KOC values, BASF believes that the appropriate KOC value to use in SCI-GROW calculations for sodium acifluorfen is either 100 or 127. However using lower than literature cited KOC values (50) and realistic half-life values, SCI-GROW still overpredicts expected residues in a conservative fashion (based on NAWQA monitoring data).

We propose the following calculation for SCI-GROW as an extremely conservative estimate.

***A Revised SCI-GROW Calculation***

| | |
|---|---|
| KOC 50 L kg | (less than any measured in the literature) |
| Half-life = 41 days | (the longest measured in the field) |
| Rate = 0.5 lb/ac x 1 | (actual label is 0.375 + 0.125 @ 14 days) |

**Ground Water Screening Concentrations in PPB = 0.401**

*If we use the same KOC chosen by EPA for surface water modeling*

| | |
|---|---|
| A KOC of 100 L kg | (used for the surface water modeling) |
| Half-life = 41 days | (the longest measured in the field) |
| Rate = 0.5 lb/ac x 1 | (actual label is 0.375 + 0.125 @ 14 days) |

**Ground Water Screening Concentrations in PPB = 0.217**

The SCI-GROW concentration using a KOC of 100 still provides a highly conservative estimate for anticipated groundwater exposure. In fact the concentrations calculated in this report are still well above the only detectable residue found in the NAWQA monitoring program. In addition, the SCI-GROW calculations presented here are in much better agreement with the data from the five retrospective ground water studies and data reported from a new prospective ground water study.

We believe that SCI-GROW provides a conservative and protective estimate of groundwater concentration when properly used. BASF believes the weight of evidence supports our choice of inputs into the model. We believe that the results calculated using our inputs provide a properly protective estimate of groundwater concentration.

**References (see Attachment for Copies of Literature Cited)**

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# Attachment A

## Copies of literature cited

J. ENVIRON. SCI. HEALTH, B29(5), 963-987 (1994)

# COMETABOLIC DEGRADATION OF ACIFLUORFEN BY A MIXED MICROBIAL CULTURE

Key words: Acifluorfen, Cometabolism, 2-Nitrobenzoate, Aminoacifluorfen.

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## ABSTRACT

Laboratory experiments were conducted to study the degradation of acifluorfen 5-[2-chloro-4-

(trifluoromethyl)-phenoxy]-2-nitrobenzoic acid by a mixed microbial population.

Concentrations of acifluorfen up to 100 mg/l had no inhibitory effect on the growth of microbial culture.

The microorganisms degraded acifluorfen through a cometabolic process in presence of 2-nitrobenzoate.

The degradation rate of acifluorfen, determined by liquid chromatography analysis in batch cultures incubated under oxygen and oxygen-limited conditions were compared. The degradation was slower under oxygen than oxygen-limited conditions. Aminoacifluorfen was produced in both conditions.

## INTRODUCTION

The extensive use of herbicides and insecticides in agriculture may be responsible for contamination of waters and soils. There, the fate of pesticide is determined by physical, chemical and biological processes. Microbiological degradation is the most important process in many cases, since it can assure a complete pesticide removal in a short time and thus preventing pollution problems. Diphenylethers are herbicides largely used in weed control in most major crops such as soybeans, rice and sugar beets crops (Hawton and Stobbe, 1971; Johnson et al., 1978), and

whose effect on weeds is to disrupt cellular and sub-cellular lipoprotein membranes in the light (Böger, 1984).

Acifluorfen, 5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-nitrobenzoic acid, is a diphenylether herbicide used for post-emergence control of broadleaf weeds in soybean production (Johnson et al., 1978).

Information regarding the persistence and degradation of acifluorfen in soil is very limited. A half-life for acifluorfen of 40 days in soil has been reported (Perucci et al., 1993). The same authors observed that the amendment of glucose increased the soil biomass and at the same time reduced the half life of acifluorfen from 40 to 28 days.

In a preliminary study Gennari et al. (1992) found that acifluorfen was degraded only in presence of 2-nitrobenzoate as co-substrate. The present study extends the investigation on the degradation of acifluorfen.

## MATERIAL AND METHODS

### Microbial culture

A mixed bacterial population capable of growing on 1 g/l of 2-nitrobenzoate as the only carbon source was used in this study.

The culture, previously obtained by enrichment culture technique, starting with an activated sludge sample from a waste-water treatment plant, was then maintained in liquid mineral medium M9 (Andreoni et al., 1990) in presence of 2-nitrobenzoate (500 mg/l) and acifluorfen (25 mg/l).

This culture was used to isolate microorganisms in pure culture. Spread plates of the mixed culture grown in presence of acifluorfen supplemented with 2-nitrobenzoate were prepared.

Different colony types were sub-cultured from these plates and assayed for their acifluorfen-degrading ability.

Growth measurements

The growth of bacteria was measured in mineral medium M9 containing either acifluorfen (25 mg/l) or acifluorfen supplemented with 2-nitrobenzoate (500 mg/l) or acetate (500 mg/l) at different intervals during the incubation period by pour plate technique.

Duplicates of 1 ml of 10-fold dilutions were plated on PCA agar (Difco). Colony counts were made after 5 days of incubation at 30°C.

Effect of herbicide on microbial growth

The effect of acifluorfen on microbial growth of the culture was tested.

The growth of bacterial culture in presence of 2-nitrobenzoate and different concentrations of acifluorfen, up to 100 mg/l, was followed by turbidity measurements made with a Klett-Summerson colorimeter (equipped with a green filter). Control was without acifluorfen.

At concentrations higher than 100 mg/l, owing to low solubility of acifluorfen, the inhibitory effect of herbicide was assayed by the standardized disk technique (Bauer et al., 1966). For plate inoculation, microorganisms were taken from cultural broths, diluted with sterile water, poured onto the culture plates containing PCA and poured off.

Sterile 12 mm paper discs, impregnated respectively with 30, 60 and 90 µl of filter sterilized solution of acifluorfen at 4% (w/v) in acetone, were then placed on the plates. The plates were kept at 4°C for one night and then incubated at 30°C. After 48 h of incubation, zones of inhibition were observed.

## Oxygen uptake measurements

Oxidative activity of microorganisms was evaluated by measuring $O_2$ uptake by resting cell suspensions, by conventional Warburg manometry at 30°C, in the presence of different oxidizable carbon sources (2-nitrobenzoate and acifluorfen). Microbial cells, grown in the maintenance conditions reported, were harvested by

centrifugation and washed three times with 0.1M potassium phosphate buffer, pH 7.0. The washed cells were resuspended in the same buffer to give an optical density of 1.2 at 540 nm. The reaction mixture in the Warburg flasks contained potassium phosphate buffer, pH 7.0, 7.32 µmoles; oxidizable source, 0.5 µmoles; washed cell suspension, 1 ml (approximately $1.2x10^8$ cells /ml) for a final volume of 3 ml. The central well contained 0.2 ml of 20% KOH.

To test the inhibitory effect of acifluorfen on respiratory activity, different µmoles of the herbicide were added to oxidizable source. All values were corrected for endogenous consumption.

Acifluorfen degradation

Experiments to determine the aerobic degradation rate of acifluorfen by growing cells of the culture were done by inoculating, with 35 ml of preculture, two series of 1000 ml-Erlenmeyer flasks each containing, in a volume of 210 ml of mineral medium M9, 25 mg/l of acifluorfen and 500 mg/l of 2-nitrobenzoate.

Stock solutions of acifluorfen at 125 mg/l and of 2-nitrobenzoate at 20 g/l, filtered through 0.22-µm-pore-size filters (Millipore Corp., Bedford, USA) were used for all experiments.

To a series of flasks the co-metabolite was added once after 7 days of incubation, to the other series,

instead, three times at a seven day interval. The cultures were incubated in the dark at 30°C on a rotary shaker.

Degradation of acifluorfen was also examined by incubating the cultures under stationary oxygen-limited conditions at 30°C in the dark. Four 500 ml-Erlenmeyer flasks each containing, in a final volume of 150 ml, 25 mg/l of acifluorfen and 500 mg/l of 2-nitrobenzoate were used for this purpose.

2-Nitrobenzoate was added once to each flask after 7 days of incubation and 3.75 mg of acifluorfen were added to two flasks after the cultures were starved for one week.

Uninoculated controls for measurements of abiotic degradation of acifluorfen was assessed in sterile medium. All treatments were done in duplicate.

Five ml of cultural medium were sampled from each flask on successive days of incubation for chemical and microbiological analyses, by using a sterile pipette.

Aliquot of the broth was diluted 1:5 with methanol, filtered on 0.2 µm regenerated cellulose membrane (Sartorius AG.W-3400, Goettingen, Germany) and then analyzed by HPLC.

HPLC conditions

HPLC was carried out on a Varian 5020 instrument equipped with a Lichrospher C18 column and UV-VIS

detector operating at 296 nm. The mobile phase 1 ml/min) was water acidified to pH 3 with orthophosphoric acid + acetonitrile (30 + 70 v/v).

Isolation and identification of acifluorfen metabolite

For isolation of the metabolite, cultural broths were acidified to pH 2 with HCl and extracted with dichloromethane. The organic phase was evapored to dryness in a rotary evaporator, the residue was diluted with acetone and subjected to preparative TLC on precoated silica gel (E. Merck, Darmstadt, Germany).

The plates were developed with a solution of chloroform/methanol/water (65:25:1 by volume).

Fluorescent areas visualized using a UV lamp (254 nm) were scraped from the plates and extracted with methanol for subsequent identification.

The identification of the metabolite was performed by mass spectrometry analysis using a Finnigan MAT 95Q instrument with magnetic electrostatic and quadrupole analyzers mounted in series.

COD determination

Chemical oxygen demands (COD) were carried out according to standard methods (APHA-AWWA-WPCF, 1971).

Samples of cultural broths were analyzed after filtration through 0.22-µm-pore-size filters.

Co-substrate utilization was monitored as the disappearance of COD. Acifluorfen residues and its transformation product were included in these COD measurements. COD values corresponding to 25 mg/l of acifluorfen and to 500 mg/l of 2-nitrobenzoate (concentrations present in mineral growth medium) were also determined.

## ATP determination

ATP content was determined on aliquots of the samples at successive incubation times. A 100 µl sample, placed into a transparent vessel, was added with 100 µl of a nucleotide releasing reagent for microbial cells (MNR, LUMAC) for ATP estraction from microbial cells and gently shaken. After 30 sec 100 µl of LUMIT reagent (luciferine + luciferase) were added and after agitation for 30 sec, the vessel was placed into a luminometer cell counter.

Luminescence was given in digital relative luminescence units (RLU) and subsequently converted into ATP content using the calibration curve obtained with pure standards (Jago et al., 1989). The luminometer used was a Biocounter P 1500 (LUMAC).

## Chemicals

The organic chemicals used for these experiments were of the highest purity available. Acifluorfen and

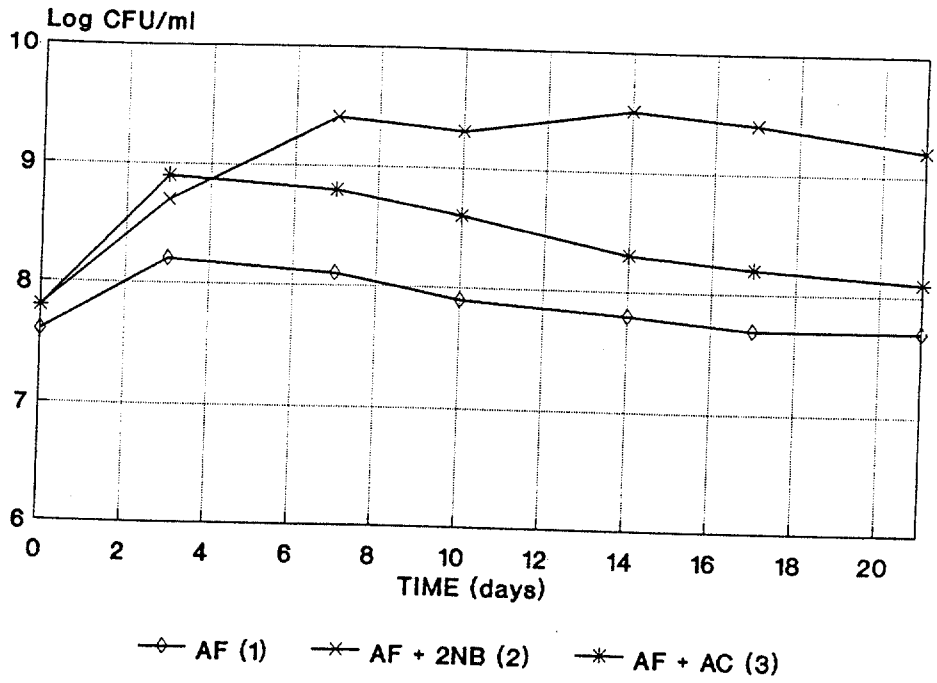


FIGURE 1

**Growth of mixed culture on mineral medium supplemented with 25 mg/l acifluorfen (1); 25 mg/l acifluorfen and 500 mg/l 2-nitrobenzoate (2); 25 mg/l acifluorfen and 500 mg/l acetate (3). CFU, Colony forming units.**

aminoacifluorfen of 97% purity were obtained from Dr. Ehrenstorfer, GmbH, Augsbourg, Germany.

## RESULTS AND DISCUSSION

### Growth measurements

The enriched microbial culture was not capable of growing when acifluorfen was added as the only carbon source. A greater growth was observed when the mixed culture was incubated in presence of 2-nitrobenzoate

than in the medium added with sodium acetate, where a low decrease of the microorganisms occurred (Fig. 1).

Eight Gram-negative and one Gram-positive strains were isolated from the culture: none of them showed any acifluorfen-degrading ability during one month of incubation, suggesting that the degradation herbicide might be done by several strains together.

## Effects of herbicide on microbial growth

Microbial population was not inhibited by the presence of 100 mg/l of acifluorfen (data not shown).

Higher concentrations of the herbicide (3.6 mg/disc) had an inhibitory effect on the growth (Fig. 2).

## Oxygen uptake experiments

Oxygen uptake exhibited by cells, grown in presence of 2-nitrobenzoate plus acifluorfen, and tested in presence of acifluorfen, 2-nitrobenzoate and 2-nitrobenzoate plus different amounts of acifluorfen is shown in Fig. 3A.

The cells did not show oxygen uptake when acifluorfen was given as oxidizable substrate; 2-nitrobenzoate was rapidly oxidized and 2-nitrobenzoate oxygenase activity was inhibited by 20.7 mg/l of acifluorfen. These results suggested to exclude the

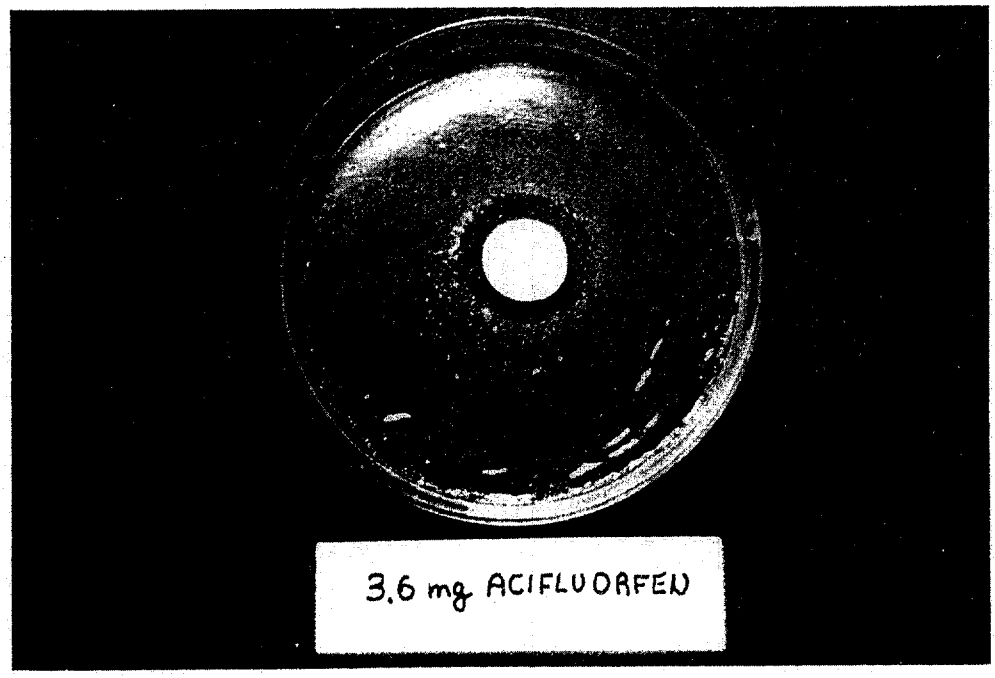


FIGURE 2

**Inhibiting effect of acifluorfen on microbial growth.**

involvement of a mechanism of oxygenation for the degradation of acifluorfen and gave evidence that 2-nitrobenzoate as growth and/or oxidizable substrate does not support acifluorfen co-oxidation activity.

Fig. 3B, referred to experiments performed with cells obtained after six months of repeated transfers at ten day intervals to the same sterile fresh medium, shows the oxidative activity of cells grown with acifluorfen, when tested in presence of acifluorfen, 2-nitrobenzoate and 2-nitrobenzoate plus acifluorfen.

OXYGEN UPTAKE (ul)
40

OXYGEN UPTAKE (ul)

FIGURE 3

Effect of acifluorfen on oxygen uptake by cells grown on 2-nitrobenzoate (2NB) and acifluorfen (AF), tested in presence of 20.7 mg/1 AF (1); 27.8 mg/1 2NB (2); 27.8 mg/1 2NB plus 8.4 mg/1 AF (3) and 27.8 mg/1 2NB plus 20.7 mg/1 AF (4). Oxygen uptake at the beginning of the experimentation (A) and after six months of repeated transfers (B). Values are corrected for endogenous oxygen uptake.

Long term exposure of microorganisms to acifluorfen did not develop a co-oxidation activity but respiratory activity of microorganisms resulted no more inhibited by the presence of 20.7 mg/l of acifluorfen.Same oxidative activity was shown by cells grown without acifluorfen (data not shown).

Degradation studies

Figures 4 and 5 present the results related to acifluorfen degradation, COD removal and ATP content over a period of 28 days, when the cultures were incubated under shaking conditions.

COD of cultural broth decreased, passing from 830-840 mg/l, values determined at the beginning of the experiment and after every addition of 2-nitrobenzoate, to 160-170 mg/l. (Figs. 4A, 5A).

As 500 mg/l of 2-nitrobenzoate dissolved in mineral medium M9 conribute to an actual COD of 760 mg/l and 25 mg/l of acifluorfen dissolved in mineral medium M9 to an actual COD of 165 mg/l, the residual COD content of culural broth could be attributed to acifluorfen or its derivative not substantially modified. 2-Nitrobenzoate, instead, could be entirely oxidized.

The possibility that 2-nitrobenzoate could be oxidized, but not acifluorfen, was consistent with data from oxygen uptake experiments.

FIGURE 4

Experimental results for acifluorfen removal in batch cultures under shaking conditions when 2-nitrobenzoate was added once on the 7th day of incubation. Values are the average of two replicates.

FIGURE 5

Experimental results for acifluorfen removal in batch cultures under shaking conditions when 2-nitrobenzoate was added three times at a seven day interval. Values are the average of two replicates.



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This hypothesis was supported by results obtained by HPLC analyses of cultural broths performed at different incubation times. While 2-nitrobenzoate was completely biodegraded in three days and no traces either of 2-nitrobenzoate or its derivatives were detected (data not shown), the degradation of acifluorfen occurred slowly and incompletely (Figs. 4B, 5B).

The amount of degraded acifluorfen did not significantly change if the supply of 2-nitrobenzoate was periodically renewed (Fig. 5B).

Acifluorfen degradation seems to require energy: ATP pool of cells, after an increase in response to the addition of 2-nitrobenzoate, lowered. The lack of ATP increase after the second addition of 2-nitrobenzoate might be due to ATP consumption to degrade acifluorfen; finally, the increase of ATP level measured after the third addition of 2-nitrobenzoate is consequent upon the further degradation of the co-substrate (Fig. 5B).

In this study ATP has been utilized as indicator of metabolic conditions. ATP is a functional cell constituent with a rapid turnover and it is know to vary depending on the metabolic activity and growth stage (Ausmus, 1973; Karl, 1980).

The disappearance of acifluorfen was accompanied by the appearance of a metabolite which was not further degraded.

Incubating microbial cultures with 2-nitrobenzoate and acifluorfen under oxygen-limited conditions, higher amounts of herbicide were degraded and about 40% of removal occurred in the first 7 days of incubation (Fig. 6). Furthermore, a more pronounced accumulation of intermediate (determined by HPLC), which gave an orange color to cultural broths, was observed.

ATP and acifluorfen contents showed comparable courses in the first 15 days of incubations. Under this condition, acifluorfen degradation was accompanied by a strong drop in ATP content of cells. The supply of 2-nitrobenzoate did not cause an increase in ATP level of microbial cells, probably because the cultures were unable to utilize immediately the co-substrate, which was instead used slower. By adding another 3.75 mg of acifluorfen to cultural broths after 14 days of incubation, the degradation of herbicide was blocked (Fig. 6B).

No degradation of acifluorfen was detected in sterile medium, indicating that abiotic transformations did not occur under our experimental conditions.

To evaluate possible herbicide adsorption onto bacterial cells, samples of cultural broths were centrifuged to separate cells. The bacterial cells were then extracted with dichloromethane and analyzed by HPLC. No adsorption of acifluorfen onto bacterial cells was detected.

FIGURE 6

Experimental results for acifluorfen removal in batch cultures under oxygen-limited conditions. (A), addition of 2-nitrobenzoate on the 7th day; (B), addition of 2-nitrobenzoate on the 7th day and acifluorfen on the 14th day. Values are the average of two replicates.

Identification of acifluorfen metabolite

A metabolite of acifluorfen was detected in HPLC at 230 nm at the retention time of 4.0 min, under the analytical conditions described above. This compound was isolated by TLC and analysed by mass spectrometry.

The electron impact mass spectrum of the metabolite and of acifluorfen are reported in figures 7 and 8. Both spectra present the fragments at m/z 196 and m/z 179 indicating that the metabolite contains the halogenated aromatic moiety of acifluorfen.

The presence of a chlorine atom is also confirmed by the typical cluster M+, M+2 and the even m/z ratio of the molecular ion of the metabolite indicates that it contains an even number of nitrogen atoms.

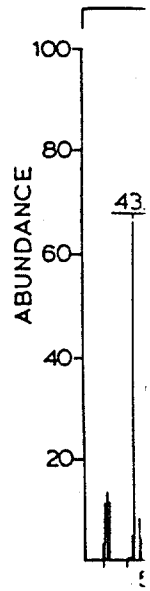
On the basis of these considerations and according to the mass of its molecular ion, the metabolite could be the aminoacifluorfen obtained by reduction of the nitro-group of acifluorfen to an amino-group.

This structure has been confirmed by mass spectrometry analysis of a pure standard of aminoacifluorfen.

## CONCLUSIONS

The results of batch studies confirm the inability of this bacterial culture to use acifluorfen as only carbon source.



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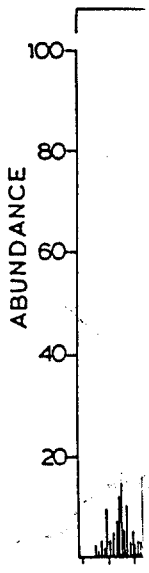

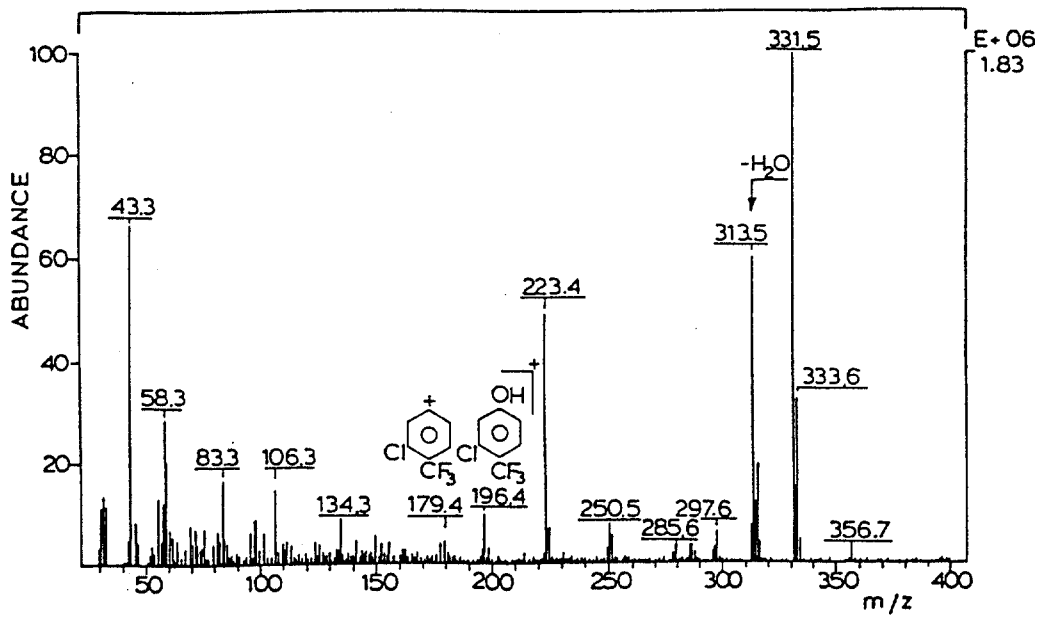


FIGURE 7

Mass spectrum of a pure standard of aminoacifluorfen.

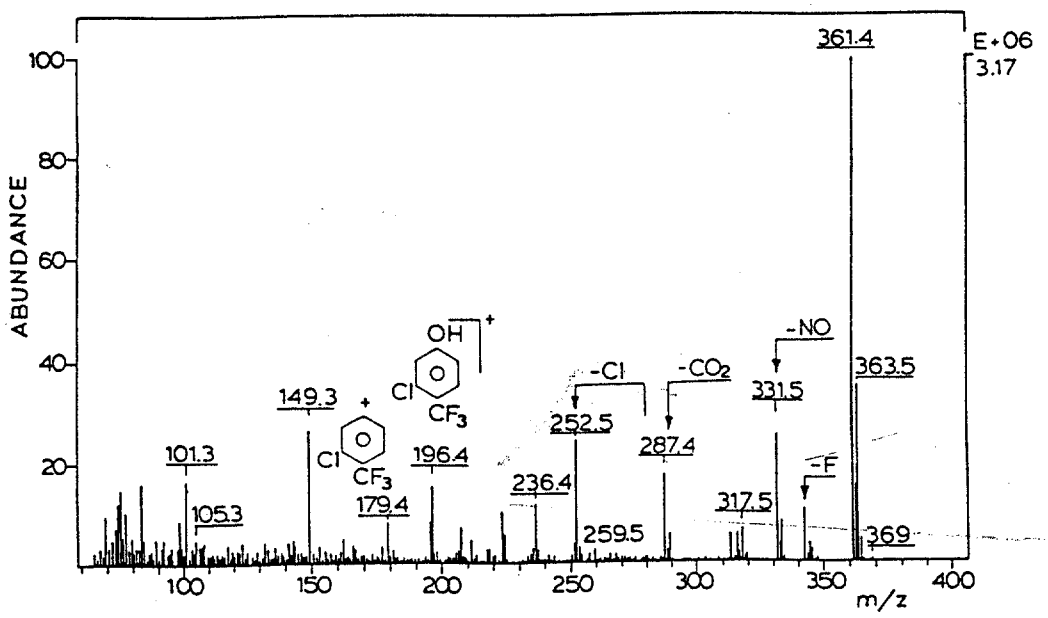


FIGURE 8

Mass spectrum of acifluorfen.

Concentrations of acifluorfen up to 100 mg/l do not inhibit the growth of microbial population; inhibition of the growth occurred only at higher concentrations.

The presence of 2-nitrobenzoate in the medium appears essential for the degradation of herbicide, for the induction of non specific enzymes of the peripheric cellular metabolism able to modify the herbicide and to generate energy, co-factors or metabolites necessary for its transformation. The capability of microorganisms to cometabolize pesticides when a biodegradable analog of the chemicals is supplemented as carbon and energy source has been demonstrated by several authors (Horvath, 1973; Engesser et al., 1988; Bollag and Liu, 1990).

While 2-nitobenzoate is completely oxidized both by growing and by resting cells, a reductive mechanism is involved in acifluorfen degradation. Degradation of acifluorfen by mixed culture does not exceed 30-40% and occurs with the formation of the corresponding aminoacifluorfen that is not further degraded.

Anaerobic conditions tend to favour reduction: degradation rate of acifluorfen under oxygen-limited conditions is faster than under shaking conditions.

Degradation of aminoacifluorfen might not proceed either for the absence of a suitable hydrogen sink needed to drive the successive degradative steps for

thermodynamic reasons (Gottschalk, 1986; Shink, 1988), or for a possible toxic effect of aminoacifluorfen on enzymes involved in the successive degradative steps.

No degradation of acifluorfen was observed with any of the nine microorganisms, as there was no loss of acifluorfen from the growth media.

Both reductive and oxidative mechanisms for the removal of nitro substituent from nitroaromatic compounds have been described and the action of nitroreductase has been demonstrated both under aerobic and anaerobic conditions (McCormick et al., 1976; Villanueva, 1964).

The xenobiotic nature of the trifluoromethyl group (TFM) (Knackmuss, 1981) and the biarylether moiety (Wittich et al., 1990) are responsible of the slow and incomplete degradation of acifluorfen. Similar findings of slow and incomplete degradation of side-chain-fluorinated aromatics have been observed for fluorodifen (Shimabukuro et al., 1982), fluormeturon (Rickard and Camper, 1978), 3-TFM-4-nitrophenol (Schultz et al., 1979) and for fluazifop (Nègre et al., 1993), whose metabolisms yielded compounds still containing TFM-group.

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# Acifluorfen Sorption, Degradation, and Mobility in a Mississippi Delta Soil

L. A. Gaston* and M. A. Locke

## ABSTRACT

**Potential surface water and groundwater contaminants include herbicides that are applied postemergence. Although applied to the plant canopy, a portion of any application reaches the soil either directly or via subsequent foliar washoff. This study examined sorption, degradation, and mobility of the postemergence herbicide acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid) in Dundee silty clay loam (fine-silty, mixed, thermic, Aeric Ochraqualf) taken from conventional till (CT) and no-till (NT) field plots. Homogeneous surface and subsurface samples were used in the sorption and degradation studies; intact soil columns (30 cm long and 10 cm diam.) were used in the mobility study. Batch sorption isotherms were nonlinear (Freundlich model) and sorption paralleled organic C (OC) content. All tillage by depth combinations of soil exhibited a time-dependent approach to sorption equilibrium that was well described by a two-site equilibrium–kinetic model. Acifluorfen degradation followed first-order kinetics. No more than about 6% of applied $^{14}$C-acifluorfen was mineralized by 49-d incubation. Extracts of incubated soil gave little indication of degradation products; however, $^{14}$C did accumulate in an unextractable fraction. Degradation was faster in the surface soils compared to subsurface soils and faster in CT surface soil compared to NT surface soil. Tillage did not affect acifluorfen degradation in subsurface samples. Elution of Br pulses from the intact soil columns under steady-state, unsaturated flow indicated preferential water flow. Nonequilibrium transport of Br was well described using a two-region, mobile–immobile water model. Inclusion of sorption kinetics in the transport model rather than assuming equilibrium sorption led to improved predictions of acifluorfen retardation. Column effluent contained negligible concentrations of acifluorfen degradation products and, as in the incubation study, an unextractable residue developed in the soil columns. However, unlike results from the incubation study, a greater fraction of applied acifluorfen was apparently bound and there was also evidence of extractable degradation products. Furthermore, first-order rate constants obtained from the batch study underestimated acifluorfen degradation during transport. Faster acifluorfen degradation in the soil columns may have been due to poorer aeration compared to the batch systems.**

THE FATE AND TRANSPORT OF POSTEMERGENCE HERBICIDES in the soil environment have received little attention. Yet these compounds come in contact with the soil, directly or as foliar washoff (Reddy et al., 1994), and are subject to leaching and runoff. Acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid) is a nitrodiphenyl ether postemergence herbicide (applied as the Na salt) that is widely used for the control of certain broadleaf weeds in soybean and peanut crops.

It exhibits a low $pK_a$ of 3.5 (Roy et al., 1983) and is highly dissociated at typical soil pHs. Despite charge repulsion effects, acifluorfen is sorbed by soil or soil constituents (Pusino et al., 1991; Ruggiero et al., 1992; Pusino et al., 1993; Gennari et al., 1994b; Nègre et al., 1995; Locke et al., 1997). Although the extent of sorption in soil is generally proportional to OC content (Gennari et al., 1994b; Nègre et al., 1995; Locke et al., 1997), sorption likely involves processes other than partitioning between aqueous and organic matter phases. In particular, acifluorfen forms complexes with divalent and trivalent cations (Pusino et al., 1991; Pusino et al., 1993) that may be sorbed or precipitated. Complex formation and subsequent sorption may partially account for increased acifluorfen sorption with decreasing soil pH or increasing cation exchange capacity (Pusino et al., 1993; Gennari et al., 1994b; Locke et al., 1997). Also, acifluorfen sorption is a nonequilibrium, time-dependent process (Locke et al., 1997). Thus, the mobility of acifluorfen in soil is affected by the rate as well as the maximum extent of sorption.

Photodegradation of nitrodiphenyl ethers may involve several different reactions including nitroreduction and hydrolysis of the ether linkage (Ruzo et al., 1980). Depending on ring substituents, dechlorination, decarboxymethylation (Ruzo et al., 1980), or, in the case of acifluorfen, decarboxylation may occur (Pusino and Gessa, 1991). Aside from photodegradation, chemical degradation of nitrodiphenyl ethers may be possible. In particular, Ohyama and Kuwatsuka (1983) found that bifenox (methyl 5-[2,4-dichlorophenoxy]-2-nitrobenzoate) underwent nitroreduction in sterilized soil. In sterile microbial culture media (Andreoni et al., 1994), however, acifluorfen does not undergo degradation. Similar to degradation data for bifenox (Ohyama and Kuwatsuka, 1983), Andreoni et al. (1994) found that acifluorfen biodegradation in microbial cultures was more rapid under anaerobic rather than aerobic conditions. Although acifluorfen biodegradation may largely be a cometabolic process (Andreoni et al., 1994), certain bacterial strains are capable of metabolizing the herbicide (Fortina et al., 1996). Degradation products of acifluorfen isolated from microbial cultures include aminoacifluorfen, 5-([2-chloro-4-(trifluoromethyl)phenyl]oxy)-2-aminobenzamide, and 5-([2-chloro-4-(trifluoromethyl)phenyl]oxy)-2-(acetylamino)benzoic acid (Gennari et al., 1994a). Aminoacifluorfen has been recovered from soil treated with acifluorfen that was incubated for 6 d (Locke et al., 1997).

The primary objective of the present study was to quantify the degradation and sorption of acifluorfen in a Mississippi Delta soil and determine whether acifluorfen fate and mobility in this soil may be accurately described on the basis of these underlying processes.

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**Abbreviations:** CT, conventional till; HPLC, high pressure liquid chromatography; NT, no-till; OC, organic carbon; TLC, thin-layer chromatography.

Sorption (isotherm and kinetics) and degradation were examined using batch systems. Intact, water-unsaturated soil columns were used to generate data on acifluorfen mobility. Because microenvironmental conditions within such soil columns may more closely match those in field soil than do conditions in batch (aerobic or anaerobic) degradation systems, data for acifluorfen mobility were also used to estimate degradation rates. A secondary objective was to assess the effects of 4 yr of no tillage (NT), compared to conventional tillage (CT), on acifluorfen degradation, sorption, and mobility.

## MATERIALS AND METHODS

### Soil

Dundee silty clay loam soil (fine-silty, mixed, thermic, Aeric Ochraqualf) was taken from CT and NT plots (subject to cotton [*Gossypium hirsutum*] cropping management) following spring cultivation (tillage operations are listed in Gaston et al. [1996]). No acifluorfen had been applied to this site for four or more years and the soils contained no detectable acifluorfen (extraction and analysis described later).

Samples included intact columns of soil (PVC pipe 10.2 cm i.d. and 30.0 cm long) and bulk soil. Collection of intact soil cores is described in Gaston and Locke (1996). Bulk samples were collected from the 0- to 10-, 10- to 20-, and 20- to 30-cm depths. Bulk soil used in the sorption experiments was air-dried, ground, mixed, and sieved (<2 mm), whereas soil for the degradation experiment was mixed and stored at 4°C until used. Chemical data for the 0- to 10-, 10- to 20-, and 20- to 30-cm depths of the CT and NT soils are given in Table 1.

### Sorption Isotherms

Five-g (oven-dry equivalent) samples of air-dry 0- to 10-, 10- to 20-, and 20- to 30-cm depth CT or NT soil were placed in 25-mL glass centrifuge tubes and 15 mL of Na acifluorfen added. The Na salt was prepared from 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid (Chem Service, West Chester, PA), as described by Roy et al. (1983). It contained 33 Bq mL$^{-1}$ $^{14}$C-ring uniformly labeled compound (99% purity, BASF, Parsippany, NJ) in 0.01 *M* $CaCl_2$ (consistent with methodology used in the mobility study, discussed below). Suspensions were shaken for 24 h. Concentrations of herbicide applied were 1, 4, 20, 100, and 500 $\mu M$ (verified by high pressure liquid chromatography [HPLC] using standards of the acid dissolved in methanol), each in triplicate. Soil solution was separated from the suspension by centrifuging (10 min at 12 000 *g*). Sorption was calculated from change in solution concentration of $^{14}$C (Tri-Carb 4000, Packard Instruments, Downers Grove, IL). Air-dry soil was used in the equilibrium and kinetic sorption studies to minimize biological activity during the course of these short-term experiments.

### Sorption Kinetics

Triplicate 5-g (oven-dry equivalent) samples of 0- to 10-, 10- to 20-, and 20- to 30-cm depth CT or NT soil were shaken with 15 mL of 10.0 $\mu M$ Na acifluorfen (containing 33 Bq mL$^{-1}$ $^{14}$C-acifluorfen) in 0.01 *M* $CaCl_2$ for periods of time up to 32 h. Soil solution was separated from the suspension as above and sorption calculated by change in solution radioactivity.

### Degradation

Twenty-five g (oven-dry equivalent) of the 0- to 10-cm CT, 0- to 10-cm NT, 20- to 30-cm CT, or 20- to 30-cm NT soils were transferred to biometer flasks (Bartha and Pramer, 1965) or 200-mL centrifuge bottles. Sufficient Na acifluorfen solution was added to each field-moist sample to supply 0.875 μmol acifluorfen (containing 5100 Bq $^{14}$C-acifluorfen) and elevate soil moisture content to 35% by weight. Ten milliliters of 1 *M* NaOH was added to the side arm of each biometer flask and the flasks closed to the atmosphere. Samples in the centrifuge bottles were extracted (procedure discussed below) within 30 min after application. Soil samples were incubated at 25°C in the dark. After 7, 14, 21, 28, 35, and 49 d, soil was quantitatively transferred to 200-mL centrifuge bottles and extracted for acifluorfen and metabolites. Each soil by sampling time combination was replicated three times. The NaOH solutions were removed from all remaining biometer flasks semiweekly and fresh NaOH added. Aliquots were analyzed for $^{14}CO_2$ by liquid scintillation counting.

**Table 1. Selected chemical properties of Dundee convention till (CT) and no-till (NT) soil at three depths.**

| Soil | Depth | Organic C¶ | pH† |
|---|---|---|---|
| | cm | % | |
| CT | 0–10 | 0.87b‡ | 5.79a |
| | 10–20 | 0.64c | 5.77a |
| | 20–30 | 0.49de | 5.80a |
| NT | 0–10 | 1.02a | 5.60a |
| | 10–20 | 0.56d | 5.26b |
| | 20–30 | 0.44e | 5.71a |

¶ Modified Mebius method (Nelson and Summers, 1982); average of three replicates.
† 1:1 soil:0.01 *M* $CaCl_2$; average of three replicates.
‡ Within a column, means followed by the same letter are not significantly different (Fisher's LSD, $\alpha = 0.05$).

Soil was extracted using 50 mL of a 60:40 methanol:water solution that was shaken for 24 h. Suspensions were centrifuged (12 min at 8000 *g*), then supernatants decanted. A 1-mL aliquot was withdrawn for $^{14}$C analysis and the remainder saved. The soil was extracted a second time for 3 h and then centrifuged. Supernatants were analyzed for $^{14}$C and combined with the prior samples. The entrained extractant was allowed to evaporate before the air-dry soil was removed, ground, and duplicate 0.3-g samples combusted (Packard Oxidizer 306, Packard Instruments) to determine unextractable $^{14}$C (corrected for contribution from the entrained solution).

Methanol in supernatants was evaporated under vacuum at 45°C (Savant Speedvac SS3, Savant Instruments, Farmingdale, NY). The resulting aqueous concentrates were acidified to pH 2 with 1 *M* HCl and passed through $C_{18}$ solid-phase extraction columns (J.T. Baker, Phillipsburg, NJ). Polar metabolites in the aqueous effluent were measured as $^{14}$C. Sorbed acifluorfen and any moderately polar metabolites were eluted from the $C_{18}$ columns with 3 mL methanol; these concentrated samples were then analyzed using an HPLC system (Waters, Milford, MA) consisting of a Maxima controler, 510 pump, 710B autosampler, and 490 UV detector. An Alltima column (Alltech, Deerfield, IL) and 60:40 acetonitrile:water (pH 3.2) eluant were used. Further details on the HPLC method are given in Locke et al. (1997). The system also included an in-line liquid scintillation counter ("-Ram detector, INUS Systems, Tampa, FL). Detection limit for acifluorfen was <0.1 $\mu M$ (50-μL injection volume).

Concentrated samples were also analyzed by thin-layer chromatography (TLC). Fifty-microliter aliquots were spotted on preadsorbent silica gel plates (20 × 20 cm, 250-μm gel, Whatman, Clifton, NJ), and developed to 10 cm using toluene:ethyl acetate:acetic acid:water (100:100:2:1, volume). The distribution of $^{14}$C was revealed using a Bioscan System 200 imaging scanner (Bioscan, Washington, DC).

## Mobility Study

The system and procedure used to establish steady-state unsaturated flow through the intact soil columns have been previously described (Gaston and Locke, 1996). Briefly, the bottom of each soil column was covered with a porous glass membrane (11.0 cm diam., Whatman) and sealed with a PVC end-plate assembly that drained into a vacuum flask. A headspace extension attached to the top of the 30-cm PVC pipe opened to a $CO_2$ trap and supported a sprinkler head. A layer of acid-washed gravel protected the soil surface from the impact of sprinkler drops.

Calcium chloride solution (0.01 *M*) was applied at constant intensity by using a metering pump (model G6 RH1, FMI, Oyster Bay, NY) and constant suction head (60 cm water) was maintained at the bottom. The application rate (1.56 cm $d^{-1}$, average for all columns) was less than the saturated conductivity of the least conductive soil columns (low end of range as determined in a preliminary study). Steady-state conditions were assumed to exist when the time-averaged change in mass of a soil column reached approximately zero.

Once apparent steady-state flow existed, the gravel was removed and 10.0-mL pulses of 1.0 *M* KBr, followed by 529 $\mu M$ Na acifluorfen (containing 4475 Bq $mL^{-1}$, $^{14}$C-acifluorfen), were uniformly applied to the soil surface (pulse and sprinkler infiltration rates equal) using glass pipets. Gravel was then replaced and sprinkler infiltration continued.

Column effluent was sampled twice daily through about two cumulative pore volumes and one daily thereafter. Aliquots were saved for Br and $^{14}$C analyses. The remainder was pooled into a series of 10 samples, each including effluent for about a 4-d flow period. Bromide was determined using ion chromatography (DX-100, DIONEX, Sunnyvale, CA). The NaOH solutions were replaced weekly and activity of trapped $^{14}CO_2$ measured.

Pooled samples were concentrated before HPLC analysis. Two hundred mL of each aqueous fraction were acidified and passed through a $C_{18}$ solid-phase extraction column as described earlier. Aqueous effluent from extraction columns was checked for polar $^{14}$C metabolites. Acifluorfen and moderately polar metabolites were then eluted with 3 mL of methanol.

The leaching experiment was terminated 40 d after application of acifluorfen and the change in mass of each column measured. The leaching apparatus was disassembled, soil carefully pushed (from bottom up) out of the PVC casing, and sectioned into 5.0-cm increments. There was no apparent soil compaction during removal. About 50-g subsamples from each section were transferred to 250-mL centrifuge bottles for extraction of acifluorfen and degradation products. Volumetric water content and bulk density with depth were calculated using the remaining soil from each section, corrected for the subsample removed. Soil subsamples were extracted as in the degradation experiment, however, with 100 mL of extractant. Aliquots of supernatant were measured for $^{14}$C activity. The entrained extracting solution was allowed to evaporate and unextractable $^{14}$C determined from duplicate 0.3-g, air-dry, ground samples upon combustion. Methanol in supernatants was evaporated and aqueous solutions concentrated and analyzed by HPLC as described above.

## Models

### Batch Systems

Sorption kinetics were described by the two-site equilibrium–kinetic model that assumes instantaneous sorption at a fraction of sites (type 1) as

$$S_1 = k_e C^N \qquad [1a]$$

where $S_1$ is sorbed concentration ($\mu$mol $kg^{-1}$), $C$ is solution concentration ($\mu M$), and $k_e$ (L $kg^{-1}$) and $N$ are empirical constants. Sorption at remaining sites (type 2) was described by $N$th-order kinetics as

$$dS_2/dt = k_f C^N - k_r S_2 \qquad [1b]$$

where $k_f$ (L $kg^{-1}$ $d^{-1}$) and $k_r$ ($d^{-1}$) are forward and reverse rate constants, respectively. Since $S = S_1 + S_2$ and $k_e + k_f/k_r = K_F$ (L $kg^{-1}$), Eq. 1a and 1b reduce to the Freundlich model, $S = K_F C^N$ at equilibrium.

The first-order kinetic model was used to describe acifluorfen degradation as

$$dM/dt = k_d M \qquad [2]$$

where $M$ is substrate mass ($\mu$mol) and $k_d$ ($d^{-1}$) is the degradation rate constant.

### Transport Systems

The mobility of acifluorfen under steady-state flow through the intact soil columns was described using the two-region model presented by van Genuchten and Wagenet (1989), which was adapted to account for variability in soil properties with depth (Gaston and Locke, 1996) and sorption kinetics:

$$\theta_{M,j}\partial C_{M,j}/\partial t + \rho_j \partial S_{M,1,j}/\partial t + \rho_j \partial S_{M,2,j}/\partial t + \theta_{IM,j}\partial C_{IM,j}/\partial t + \rho_j \partial S_{IM,1,j}/\partial t + \rho_j \partial S_{IM,2,j}/\partial t = \theta_{M,j} D_j \partial^2 C_{M,j}/\partial z^2 - \theta_{M,j} v_j \partial C_{M,j}/\partial z - F(_{M,j}, S_{M,j}) \qquad [3a]$$

$$\theta_{IM,j}\partial C_{IM,j}/\partial t + \rho_j \partial S_{IM,1,j}/\partial t + \rho_j \partial S_{IM,2,j}\partial t = \alpha_j (C_{M,j} - C_{IM,j}) - G(C_{IM,j}, S_{IM,j}) \qquad [3b]$$

where subscripts M and IM refer to mobile and immobile water regions, respectively; subscripts 1 and 2 refer to equilibrium- and kinetic-type sorption sites, respectively; subscript $j$ denotes the $j^{th}$ of $N$ depth increments; $\theta$ is volumetric water content; $\rho$ is bulk density (Mg $m^{-3}$); $D$ is the dispersion coefficient ($cm^2$ $d^{-1}$); $v$ is pore water velocity (cm $d^{-1}$); $\alpha$ is the mass transfer coefficient for diffusion between mobile and immobile water regions ($d^{-1}$); $t$ is time (d); and $z$ is depth (cm). The functions $F$ and $G$ describe acifluorfen degradation in the mobile and immobile water regions, respectively.

Boundary and initial conditions were

$$-D_1 \partial C_{M,1}/\partial z + v_1 C_{M,1} = v_1 C_0 \qquad 0 < t \le t_p,\ z = 0 \qquad [3c]$$

$$-D_1 \partial C_{M,1}/\partial z + v_1 C_{M,1} = 0 \qquad t > t_p,\ z = 0 \qquad [3d]$$

$$\theta_{M,j} D_j \partial C_{M,j}/\partial z = \theta_{M,j-1} D_{j-1} \partial C_{M,j-1}/\partial z \qquad t > 0,\ z = jL/N \qquad [3e]$$

$$\partial C_{M,N}/\partial t = 0 \qquad t > 0,\ z = L \qquad [3f]$$

$$C_{M,j} = 0 \qquad t = 0,\ 0 \le z \le L \qquad [3g]$$

$$C_{IM,j} = 0 \qquad t = 0,\ 0 \le z \le L \qquad [3h]$$

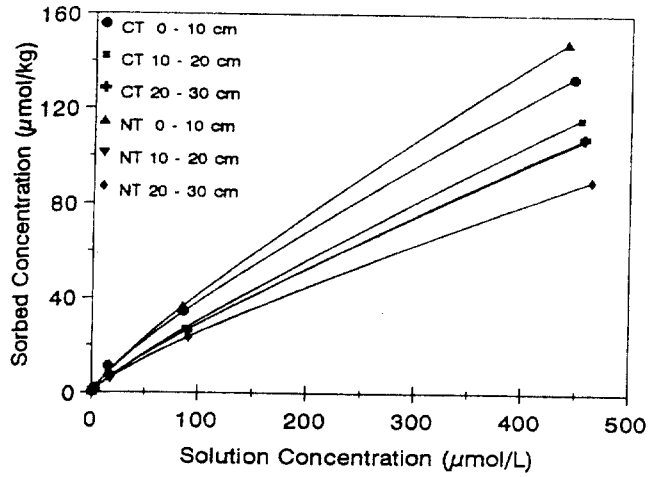


**Fig. 1. Acifluorfen sorption isotherms for three depth increments in Dundee conventional till (CT) and no-till (NT) soil. Curves show best-fits of the Freundlich model to each experimental isotherm.**

where $C_0$ is concentration of the input pulse (μmol $L^{-1}$), $L$ is column length (cm), and $t_p$ is pulse duration (d).

The two-site sorption model for mobile and immobile water regions was

$$S_{M,1,j} = f_j k_{e,j}(C_{M,j})^{N_j} \quad [4a]$$

$$\partial S_{M,2,j}/\partial t = f_j k_{f,j}(C_{M,j})^{N_j} - k_{r,j} S_{M,2,j} \quad [4b]$$

$$S_{IM,1,j} = (1 - f_j) k_{e,j}(C_{IM,j})^{N_j} \quad [4c]$$

$$\partial S_{IM,2,j}/\partial t = (1 - f_j) k_{f,j}(C_{IM,j})^{N_j} - k_{r,j} S_{IM,2,j} \quad [4d]$$

where $f_j$ is the fraction of sorption sites in the mobile water region of depth increment $j$. This fraction was assumed proportional to the fraction of mobile water, $f_j = \theta_{M,j}/\theta_j$.

If sorption kinetics are ignored, Eq. 4a–4d reduce to corresponding Freundlich models for mobile and immobile water regions written as

$$S_{M,1,j} = f_j K_j (C_{M,j})^{N_j} \quad [4e]$$

$$S_{IM,1,j} = (1 - f_j) K_j (C_{IM,j})^{N_j} \quad [4f]$$

No attempt was made to distinguish between degradation occurring in solution and possibly in the sorbed phase; rather, these processes were lumped, so that

$$F(C_{M,j}, S_{M,j}) = k_{M,j}(\theta_{M,j} C_{M,j} + \rho_j [S_{M,1,j} + S_{M,2,j}]) \quad [5a]$$

$$G(C_{IM,j}, S_{IM,j}) = k_{IM,j}(\theta_{IM,j} C_{IM,j} + \rho_j [S_{IM,1,j} + S_{IM,2,j}]) \quad [5b]$$

where $k_{M,j}$ and $k_{IM,j}$ ($d^{-1}$) are first-order degradation rate constants for the two regions in depth increment $j$.

Additionally, $\theta_{M,j}$ was assumed to be directly proportional to total volumetric water content, $\theta_j$ ($\theta_{M,j}/\theta_j = \theta_M/[\theta_{M,j} + \theta_{IM,j}] = \varepsilon$), and $D_j$ and $\alpha_j$ directly proportional to pore water velocity, $v_j$ ($D_j/v_j = D_j\theta_j/q = \Psi$ [cm]; $\alpha_j/v_j = \alpha_j\theta_j/q = \phi$ [$cm^{-1}$]). Justifications for these assumptions have been discussed in Gaston and Locke (1996). The parameters ε, ϕ, and ψ were determined from tracer elution curves. Bulk density $\rho_j$ and total volumetric water content $\theta_j$ were determined from masses of column sections before and after drying.

Approximate solutions were generated using an implicit finite difference method. A least-squares procedure (van Genuchten, 1981), coupled with either the transport model (Eq. [3]–[5]) or batch sorption kinetics model (Eq. [2a] and [2b]), was used for parameter estimation.

**Table 2. Sorption isotherm and two-site equilibrium–kinetic model parameters for Dundee conventional till (CT) and no-till (NT) soil at three depths.**

| Soil | Depth | Sorption parameter | | | | |
|---|---|---|---|---|---|---|
| | | $K_F$ | $N$ | $k_e$ | $k_f$ | $k_r$ |
| | cm | L kg$^{-1}$ | | L kg$^{-1}$ | L kg$^{-1}$ d$^{-1}$ | d$^{-1}$ |
| CT | 0–10 | 0.83 ± 0.04¶ | 0.82 ± 0.01 | 0.57 ± 0.02 | 0.82 ± 0.34 | 3.0 ± 1.4 |
| | 10–20 | 0.52 ± 0.05 | 0.87 ± 0.02 | 0.44 ± 0.01 | 0.11 ± 0.09 | 0.7 ± 1.5 |
| | 20–30 | 0.54 ± 0.02 | 0.85 ± 0.01 | 0.40 ± 0.02 | 0.36 ± 0.30 | 2.4 ± 2.4 |
| NT | 0–10 | 0.80 ± 0.01 | 0.85 ± 0.01 | 0.58 ± 0.02 | 0.43 ± 0.21 | 1.6 ± 1.1 |
| | 10–20 | 0.54 ± 0.02 | 0.85 ± 0.01 | 0.41 ± 0.02 | 0.22 ± 0.18 | 1.1 ± 1.6 |
| | 20–30 | 0.56 ± 0.01 | 0.81 ± 0.01 | 0.37 ± 0.02 | 0.71 ± 0.37 | 3.6 ± 2.0 |

¶ Standard error.

# RESULTS AND DISCUSSION

## Sorption Isotherms

Freundlich models for acifluorfen sorption (Fig. 1) indicated greater sorption in the Dundee NT compared with the CT surface soil. This is consistent with the slightly greater OC content of the NT soil (Table 1). In general, the extent of acifluorfen sorption paralleled OC content, decreasing with increasing depth below the soil surface. Also, greater sorption in the CT subsurface, compared with the NT subsurface (10 to 20 cm and 20 to 30 cm), was consistent with slightly higher OC content in the CT soil samples. However, differences due to tillage at the three depths were sufficiently small and uncertainty in Freundlich parameters (Table 2) sufficiently high that models for sorption in CT and NT soil at any depth were not significantly different (Taylor series approximation method of Hinds and Milliken [1987]; data not shown). On the other hand, comparison of sorption at each initial acifluorfen concentration (Table 3) shows that sorption was generally greater in the NT surface soil than in the corresponding CT soil and that sorption was generally greater in the subsurface CT soils than in corresponding NT soils.

## Sorption Kinetics

Sorption of acifluorfen in all depths of the CT and NT Dundee soils was time-dependent and well described by the two-site kinetic–equilibrium model. Figures 2a–2c show effect of tillage on extent of sorption (fraction of maximum based on $K_F$ calculated from fitted parameters $K_F = k_e + k_f/k_r$) as a function of reaction time in the 0- to 10-, 10- to 20-, and 20- to 30-cm depths (parameter values given in Table 2). Also shown are best-fits of the

**Table 3. Acifluorfen sorption in Dundee conventional till (CT) and no-till (NT) soil at three depths and five initial acifluorfen concentrations.**

| Soil | Depth | Initial acifluorfen concentration (μ*M*) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 4 | 20 | 100 | 500 |
| | cm | μmol/kg | | | | |
| CT | 0–10 | 0.57a¶ | 1.8b | 7.8b | 32.9a | 125.7b |
| | 10–20 | 0.46c | 1.6c | 7.2b | 25.1b | 105.7c |
| | 20–30 | 0.49b | 1.6c | 6.5c | 24.1bc | 96.9c |
| NT | 0–10 | 0.48bc | 2.0a | 8.6a | 35.1a | 141.2a |
| | 10–20 | 0.41d | 1.5cd | 6.4c | 24.6b | 98.9c |
| | 20–30 | 0.39d | 1.4d | 5.6d | 21.6c | 80.5d |

¶ Within a column, means followed by the same letter are not significantly different (Fisher's LSD, α = 0.05).

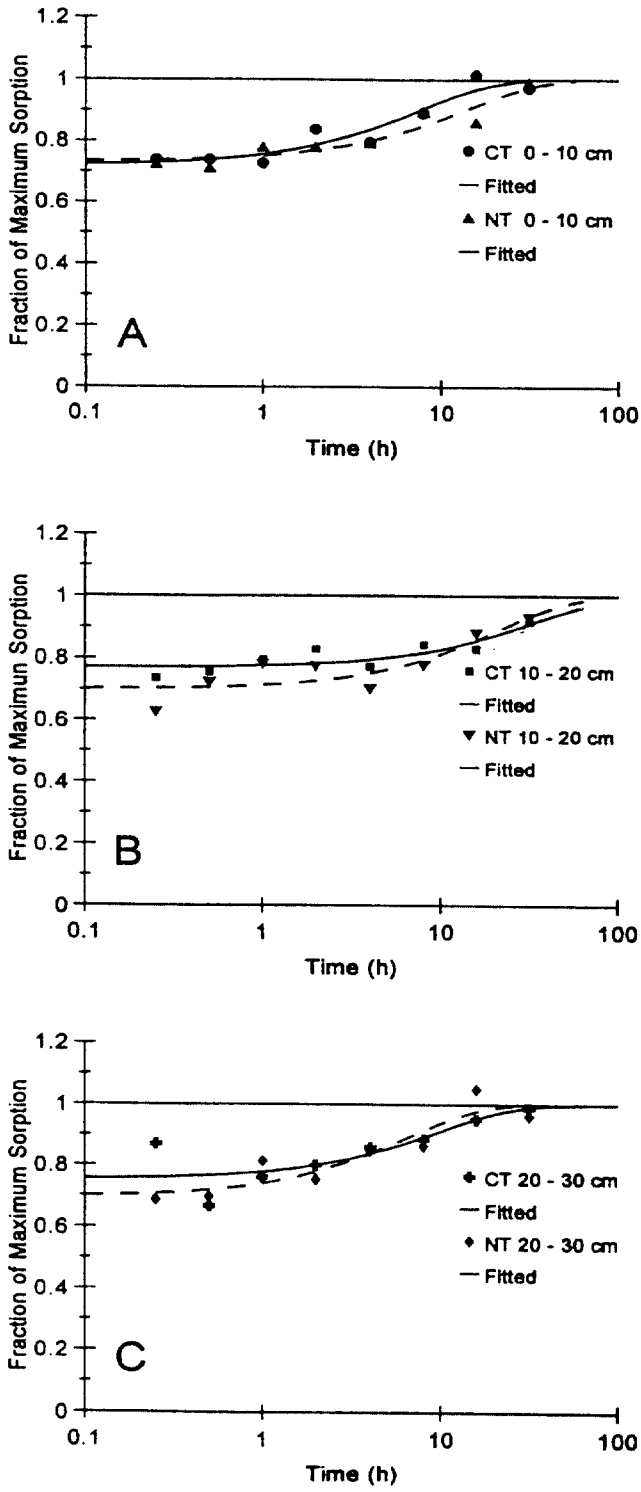


**Fig. 2. Acifluorfen sorption kinetics for: (A) 0–10 cm, (B) 10–20 cm, and (C) 20–30 cm depth increments in Dundee conventional till (CT) and no-till (NT) soil. Curves show best-fits of the two-site equilibrium–kinetic model to each time-course data set. All data are relative to equilibrium sorption.**

two-site model. Comparison of these scaled data reveals little or no differences among the soils in extent of instantaneous sorption (average $k_e/K_F \sim 0.7$). Because of fairly large uncertainty in estimations of $k_f$ and $k_r$ (Table 2), the effect (if any) of tillage on acifluorfen sorption kinetics is unclear. It is apparent from Fig. 2a–2c, however, that equilibrium was generally not achieved by 24-h contact time.

## Degradation

Despite up to 40% degradation of acifluorfen during the 49-d experiment (Table 4), chromatograms of the concentrated $^{14}C$ extracts gave no evidence of metabolites; corrected for background, $^{14}C$-acifluorfen accounted for essentially all (average > 99%) radioactivity in the HPLC and TLC chromatograms. In particular, no extractable aminoacifluorfen was found. Furthermore, the solid-phase extraction procedure recovered >99% of initially extracted $^{14}C$. Thus, any polar metabolites (unrecovered in this step), along with any less-polar metabolites retained by the $C_{18}$ extraction columns, constituted a very minor fraction of extracted $^{14}C$.

Degradation of acifluorfen was accompanied by development of an unextractable fraction of $^{14}C$ (Table 4). Recent work by Locke et al. (1997) showed that aminoacifluorfen exhibited high sorption affinity ($K_F$ > 40 L kg$^{-1}$) in a Dundee soil, especially at low concentrations (highly nonlinear, with $N = 0.41$), and that less than 10% of the applied aminoacifluorfen was extractable after 24-h contact. Similar reactivity of aminoacifluorfen in the Dundee CT and NT soils may account for absence of this metabolite and, in part, contribute to accretion of the unextractable fraction of $^{14}C$. Based on previous work (Locke et al., 1997), binding of acifluorfen to soil colloids seems unlikely. Although short-term sorption kinetics were well-described by the two-site equilibrium–kinetic model, continued sorption of $^{14}C$ beyond about 48 h could not be accounted for by assuming irreversible sorption of acifluorfen. However, the time-dependent increase in $^{14}C$ could be described by assuming irreversible sorption of acifluorfen degradation products (Locke et al., 1997).

Slow mineralization of acifluorfen was consistent with negligible concentration of extractable degradation intermediates. Despite the generally slow rate of evolution of ring-$^{14}C$ as radio-labeled $CO_2$, the data revealed differences among the four soils due to tillage and depth below the soil surface (Table 4). Mineralization was generally faster in the surface soils than in the corresponding subsurface soils and faster in the 0- to 10-cm CT soil than in the corresponding NT surface soil. Relative mineralization among the four soils was consistent with extent of acifluorfen degradation and accumulation of unextractable $^{14}C$ (Table 4).

Acifluorfen degradation was generally greater in the surface soils for incubation times beyond 14 d (Table 4). By Day 21, degradation was generally greater in the surface CT than in the respective NT soil. There was no effect due to tillage in the subsurface soils. Acifluorfen degradation was described by first-order kinetics (Eq.

**Table 4. Recovery of acifluorfen and $^{14}C$ applied in the acifluorfen degradation study.**

| Data set | Soil | Depth | Incubation time (d) 7 | 14 | 21 | 28 | 35 | 49 |
|---|---|---|---|---|---|---|---|---|
| | | cm | % of applied | | | | | |
| Acifluorfen | CT† | 0–10 | 93.0a¶ | 83.7b | 78.5c | 78.6c | 77.8c | 61.0b |
| | NT‡ | 0–10 | 94.2a | 87.8b | 88.4b | 87.4b | 80.0bc | 72.0ab |
| | CT | 20–30 | 93.8a | 95.6a | 91.6a | 92.6a | 85.3ab | 80.9a |
| | NT | 20–30 | 93.7a | 86.9b | 91.6a | 93.9a | 87.7a | 80.4a |
| Mineralized | CT | 0–10 | 1.8a | 3.2a | 4.0a | 4.4a | 5.6a | 6.2a |
| | NT | 0–10 | 1.5b | 2.7b | 3.3b | 4.0b | 4.3b | 5.2b |
| | CT | 20–30 | 1.4c | 2.4c | 3.0c | 3.5c | 4.2b | 4.7b |
| | NT | 20–30 | 1.3c | 2.1d | 2.7d | 3.2d | 3.7c | 4.1c |
| Unextractable | CT | 0–10 | 5.9a | 7.6a | 11.0a | 10.9a | 12.2a | 12.1a |
| | NT | 0–10 | 3.8b | 5.9b | 7.2b | 8.8a | 9.7a | 9.4b |
| | CT | 20–30 | 1.6c | 1.3c | 2.7c | 3.2b | 3.0b | 3.4c |
| | NT | 20–30 | 1.7c | 2.5c | 3.5c | 4.6b | 5.0b | 4.7c |
| Total $^{14}C$ | CT | 0–10 | 99.7 | 94.5 | 93.5 | 93.9 | 95.6 | 79.3 |
| | NT | 0–10 | 99.5 | 96.4 | 94.9 | 100.2 | 94.0 | 86.6 |
| | CT | 20–30 | 96.8 | 99.3 | 97.3 | 99.3 | 92.5 | 89.0 |
| | NT | 20–30 | 96.7 | 91.5 | 97.8 | 100.7 | 96.5 | 89.2 |

¶ In data set, within a column, means followed by the same letter are not significantly different (Fisher LSD, $\alpha = 0.05$).
† CT, conventional till.
‡ NT, no-till.

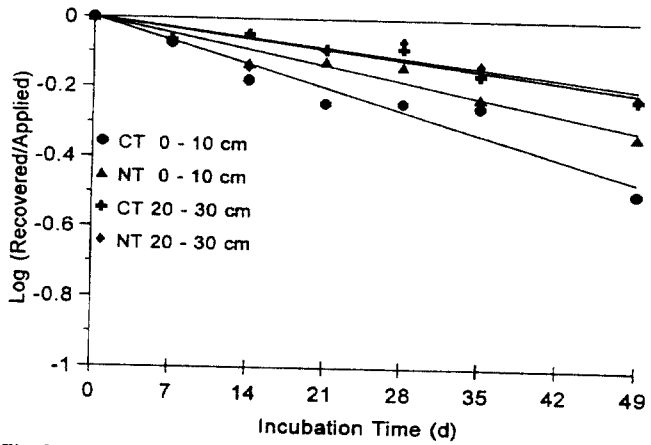


**Fig. 3. Degradation kinetics of acifluorfen in surface and subsurface Dundee conventional till (CT) and no-till (NT) soil. Lines show best-fits of the first-order model to each experimental data set.**

[2]), as shown in Fig. 3. The modeled data indicate faster degradation in the CT surface soil than in corresponding NT soil, faster degradation in the surface soils than in subsurface soils, but no difference due to tillage in the subsurface soils. Rate constants are given in Table 5.

Faster degradation in the surface soils compared with subsurface soils was consistent with greater metabolic activity and high numbers of microorganisms in the surface soils (Table 2, Gaston et al., 1996). However, faster acifluorfen degradation in the CT, compared with NT, surface soil (Fig. 3 and Tables 4 and 5) would not have been expected on the basis of microbiological data that indicated no difference in degradation potential. On the other hand, slightly greater acifluorfen sorption in the NT surface soil might result in slower degradation if degradation were limited to solution phase substrate. However, fits of the two-site model (Eq. [1a] and [1b]), extended to include solution phase first-order degradation of acifluorfen and irreversible sorption of degradation product(s) (Locke et al., 1997) to data for recovered acifluorfen and unextractable $^{14}C$, failed to account for the inconsistency between degradation and microbial data. The degradation rate constant estimated for the CT soil remained larger (CT $k_d = 0.025 \pm 0.02$ and NT $k_d = 0.018 \pm 0.01$) due to only slightly greater sorption in the NT surface soil. Apparently, the greater rate of acifluorfen degradation (and mineralization) in the CT surface soil simply reflected a larger population of microorganisms capable of degrading acifluorfen.

## Preferential Flow through Intact Soil Columns

All Br elution curves indicate some degree of preferential flow. Examples in Fig. 4 for columns CT 1 and NT 2 show a range of Br peak concentrations from about 0.2 to 0.4 pore volumes earlier, respectively, than expected in the absence of preferential flow. The mobile–immobile water model (Eq. [3], without sorption or degradation) was capable of providing good descriptions of Br elution in all cases. Optimized parameters are given in Table 6. Measured variation in soil bulk density and water content with depth below the surface of each soil column is shown in Table 7. Small $\phi$ values (Table 6) indicate slow mass transfer between mobile and immobile water regions.

## Acifluorfen Mobility

### Experimental Results

Chromatograms of soil column effluents revealed no $^{14}C$ peaks other than for $^{14}C$-acifluorfen (corrected for background, radioactivity eluting with acifluorfen accounted for ave. 99% in chromatograms). Less than 2% effluent $^{14}C$ passed through the $C_{18}$ extraction columns used in HPLC sample preparation, indicating low concentration of polar $^{14}C$-compounds. There was loss of <2% of the effluent $^{14}C$ during solid-phase extraction.

**Table 5. First-order rate constants for acifluorfen degradation obtained from batch and transport data.**

| Soil | Depth | Batch $k_d$ | Transport $k_d$ from soil column CT 1 | CT 2 | NT 1 | NT 2 |
|---|---|---|---|---|---|---|
| | cm | | $d^{-1}$ | | | |
| CT† | 0–10 | 0.0094 ± 0.0007¶ | 0.045 ± 0.006 | 0.054 ± 0.011 | | |
| | 20–30 | 0.0041 ± 0.0004 | 0.060 ± 0.009 | 0.016 ± 0.004 | | |
| NT‡ | 0–10 | 0.0064 ± 0.0004 | | | 0.059 ± 0.006 | 0.096 ± 0.012 |
| | 20–30 | 0.0042 ± 0.0009 | | | 0.011 ± 0.004 | 0.002 ± 0.001 |

¶ Standard error.
† CT, conventional till.
‡ NT, no-till.

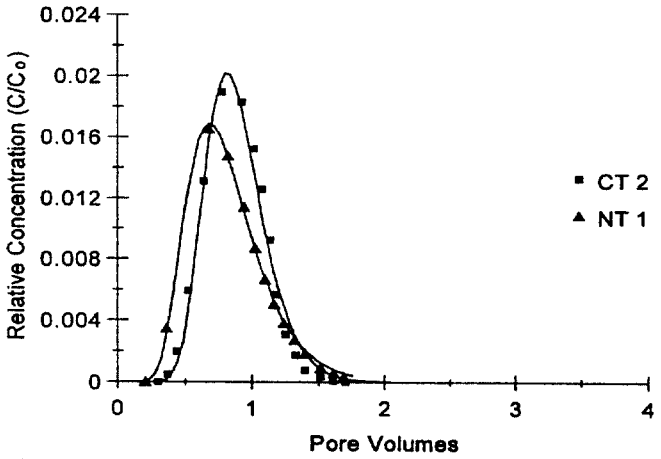


**Fig. 4. Example Br elution curves from two intact soil columns showing the effect of preferential water flow. Curves are best-fits of the two-region mobile–immobile water model.**

Therefore, $^{14}$C-acifluorfen accounted for about 97% of the radioactivity in column effluent and $^{14}$C effluent concentrations may be taken as proportional to effluent acifluorfen concentrations. Figure 5 shows average effluent concentrations of $^{14}$C-acifluorfen for the CT and NT soil columns. Acifluorfen elution from the NT columns was slightly delayed compared to elution from the CT columns.

Although there was little evidence for acifluorfen degradation products in the soil column effluent, about 39% of the $^{14}$C extracted from the soil columns could not be attributed to acifluorfen. Due to low total radioactivity in these extracts, however, the only obvious chromatographic peak was $^{14}$C-acifluorfen. Of the total $^{14}$C extracted, about 26% was not retained by the $C_{18}$ columns and 13% unrecovered in either HPLC samples or as polar compounds in aqueous effluent from the $C_{18}$ columns. The concentration of degradation products was greatest at the soil surface (0–5 cm depth segment). About 11% of the applied acifluorfen was recovered from the NT soil columns by extraction, whereas 9% was extracted from the CT columns. Slightly greater solute retardation in the NT soil columns (Fig. 5) may account for this small difference in acifluorfen recovery. Table 8 shows $^{14}$C recovered as leachate, soil extract, and liberated upon combustion.

Data on sorption affinity (or poor extractability) of acifluorfen degradation products such as aminoacifluorfen (Locke et al., 1997) suggest that development of an unextractable fraction reflects degradation of acifluorfen to more highly sorbed compounds. If this is the case, slightly greater accumulation of unextractable $^{14}$C in the CT soil columns (34% of applied compared to 27% in the NT columns) may indicate a somewhat faster rate of acifluorfen transformation in the CT soil, consistent with biometer flask data for the 0- to 10-cm depth soils (Tables 3 and 4). Also, average sum of unextractable $^{14}$C plus extractable degradation products was higher in the CT soil (39% compared with 35%). However, lower recovery of degradation products from the NT soil columns may reflect lower average total recovery of $^{14}$C (Table 8).

**Table 7. Volumetric water content (θ) and bulk density (ρ) at various depths in the Dundee soil columns.**

| | Column | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CT† 1 | | CT 2 | | NT‡ 1 | | NT 2 | |
| Depth | θ | ρ | θ | ρ | θ | ρ | θ | ρ |
| cm | | Mg m$^{-3}$ | | Mg m$^{-3}$ | | Mg m$^{-3}$ | | Mg m$^{-3}$ |
| 0–5 | 0.358 | 1.11 | 0.335 | 1.10 | 0.398 | 1.22 | 0.369 | 1.07 |
| 5–10 | 0.444 | 1.51 | 0.403 | 1.45 | 0.390 | 1.41 | 0.390 | 1.44 |
| 10–15 | 0.402 | 1.49 | 0.405 | 1.53 | 0.392 | 1.54 | 0.400 | 1.52 |
| 15–20 | 0.409 | 1.47 | 0.387 | 1.43 | 0.384 | 1.53 | 0.371 | 1.57 |
| 20–25 | 0.417 | 1.48 | 0.400 | 1.48 | 0.390 | 1.53 | 0.376 | 1.59 |
| 25–30 | 0.410 | 1.44 | 0.356 | 1.48 | 0.396 | 1.51 | 0.364 | 1.58 |

† CT, conventional till.
‡ NT, no-till.

## Simulation Results

Bromide elution curves indicated that water flow through the intact soil columns bypassed a portion of the total pore water volume (Fig. 4). Results of the batch sorption study showed that acifluorfen sorption in Dundee CT and NT soils is time-dependent (Fig. 2a–2c). Thus, it seems possible that sorption kinetics as well as preferential water flow might affect acifluorfen transport. Figure 6 shows $^{14}$C-acifluorfen effluent concentrations for column CT 1 compared to simulations assuming either instantaneous sorption equilibrium (Eq. [4e]–[4f]) or time-dependent sorption (Eq. [4a]–[4d]). Sorption parameters given in Table 2 were used. Inclusion of sorption kinetics led to a better description of acifluorfen retardation (Fig. 6). However, the fairly slow pore water velocity (Table 5) and long solute residence time favored close approach to sorption equilibrium; ignoring sorption kinetics resulted in <10% error in predicted retardation (Fig. 6).

In general, use of the batch sorption kinetics data in the transport model led to accurate prediction of acifluorfen mobility through the intact soil columns. Fig-

**Table 6. Transport model parameters for the Dundee soil columns.**

| | Column | | | |
|---|---|---|---|---|
| Parameter | CT† 1 | CT 2 | NT‡ 1 | NT 2 |
| r (=$\theta_{im}/\theta$) | 0.81 ± 0.01 | 0.88 ± 0.01 | 0.82 ± 0.01 | 0.94 ± 0.01 |
| ω (=$\alpha\theta/q$, cm ) | 0.001 ± 0.001 | 0.001 ± 0.002 | 0.001 ± 0.001 | 0.001 ± 0.001 |
| ψ (=$D\theta/q$, cm) | 1.10 ± 0.03 | 0.96 ± 0.08 | 1.96 ± 0.05 | 2.6 ± 0.2 |
| q (cm d$^{-1}$) | 1.53 | 1.53 | 1.67 | 1.57 |
| $t_p$ (d) | 0.080 | 0.080 | 0.076 | 0.080 |

† CT, conventional till.
‡ NT, no-till.

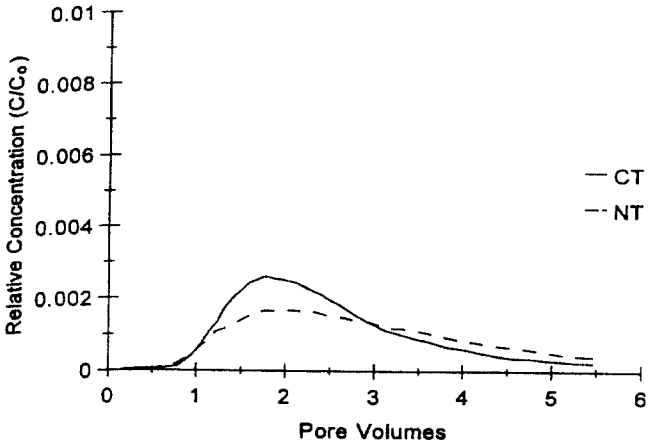


**Fig. 5. Effect of tillage on elution of acifluorfen pulses applied to the surface of intact columns of Dundee conventional till (CT) and no-till (NT) soil. Average relative concentrations are shown.**

ure 7a presents average measured and predicted acifluorfen effluent concentrations for the four soil columns. Although the batch sorption data were clearly appropriate for predicting volume of water required to displace acifluorfen, the batch degradation data substantially underestimated the rate of acifluorfen degradation in the soil columns. Comparison of measured and predicted distribution of unextractable (apparently bound) $^{14}C$ reveals a similar discrepancy.

Based on results from the degradation and mobility studies, intermediate products of acifluorfen degradation in the Dundee soils apparently exhibit high affinity for sorption. Thus, to a first approximation, such compounds may be assumed immobile (within the time scale of the transport experiment) and to accumulate in place where formed. Concentrations of acifluorfen degradation products predicted using Eq. [5a] and [5b] may be compared to the sum of extractable degradation products plus unextractable $^{14}C$. Figure 7b shows this comparison the for upper, middle, and lower 10-cm soil segments. Concentrations predicted on the basis of batch data were smaller, indicating that acifluorfen degradation proceeded at a faster rate in the soil columns than in the batch systems.

To quantify the magnitude of error in batch degradation rate constants that was responsible for the discrepancies between predicted and measured data (Fig. 7a and 7b), rate constants were adjusted to fit the acifluorfen effluent concentration and residual $^{14}C$ data. First-order degradation was assumed and rate constants for the top and bottom 10-cm segments were optimized to obtain best-fits to the experimental data. The rate constant for the middle segment was assumed to be the average of these values. Also, based on the results of Gaston and Locke (1996), there was little to be gained by trying to estimate different rate constants for mobile and immobile water regions because mass transfer between these regions was slow (Table 6). Calculated first-order degradation rate constants for the four soil columns are listed in Table 5.

Estimated rate constants were often nearly an order

**Table 8. Recovery of $^{14}C$ applied to the Dundee soil columns.**

| Column | Fraction | | | |
|---|---|---|---|---|
| | Effluent | Extractable | Unextractable | Total |
| | % of $^{14}C$ applied | | | |
| CT* 1 | 45.7 | 16.7 | 30.0 | 92.4 |
| CT 2 | 57.1 | 10.1 | 38.4 | 105.5 |
| NT† 1 | 55.3 | 14.0 | 23.4 | 92.7 |
| NT 2 | 35.0 | 23.7 | 31.0 | 89.7 |

* CT, conventional till.
† NT, no-till.

of magnitude greater than the corresponding values obtained from the biometer flask study. Consistent with the batch data, however, rate constants for the surface soil were generally greater than those for the 20- to 30-cm depth. Adequacy of fits to the NT 1 column data are shown in Fig. 8a and 8b. The mass of acifluorfen displaced through the soil column was accurately described (Fig. 8a) and distribution of residual $^{14}C$ better estimated (Fig. 8b) than when batch degradation data were used.

Since acifluorfen degradation is more rapid under anaerobic conditions (Andreoni et al., 1994), greater degradation rates in the soil columns may reflect poorer aeration than in the biometer flasks. In contrast to the batch systems, which each contained a thin layer of 25-g soil and were vented when NaOH was replaced, only the top surface of the soil columns was exposed to the atmosphere (directly, during replacement of NaOH, or indirectly, via sprinkler application of aerated 0.01 *M* $CaCl_2$ solution). Thus, the ratio of external surface area to internal volume was much larger and path length for $O_2$ diffusion much shorter in the batch systems.

Because intact soil columns are approximate models for undisturbed soil, the distribution of $k_d$ values with depth may be similar to field values under similar moisture conditions (wet, though unsaturated, with an average 0.07 void space, as calculated from the data in Table 7, assuming soil particle density of 2.65 Mg $m^{-3}$). Therefore, $k_d$s obtained from the mobility study may tend

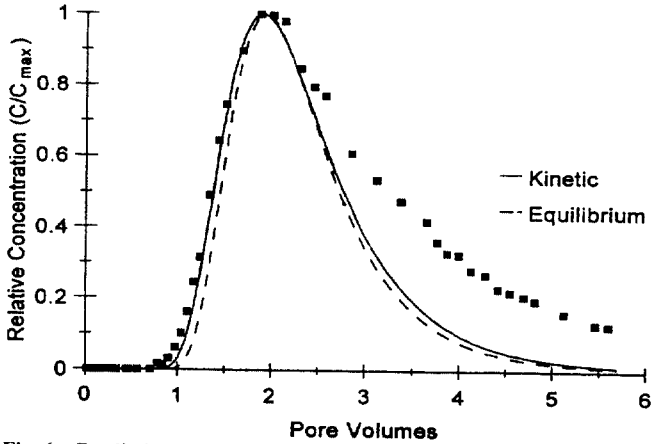


**Fig. 6. Predictions of acifluorfen elution from intact conventional till (CT) soil column 1 obtained when allowing for sorption kinetics or assuming sorption equilibrium compared with measured effluent concentrations. All concentrations in a curve are relative to the maximum concentration in that data set.**

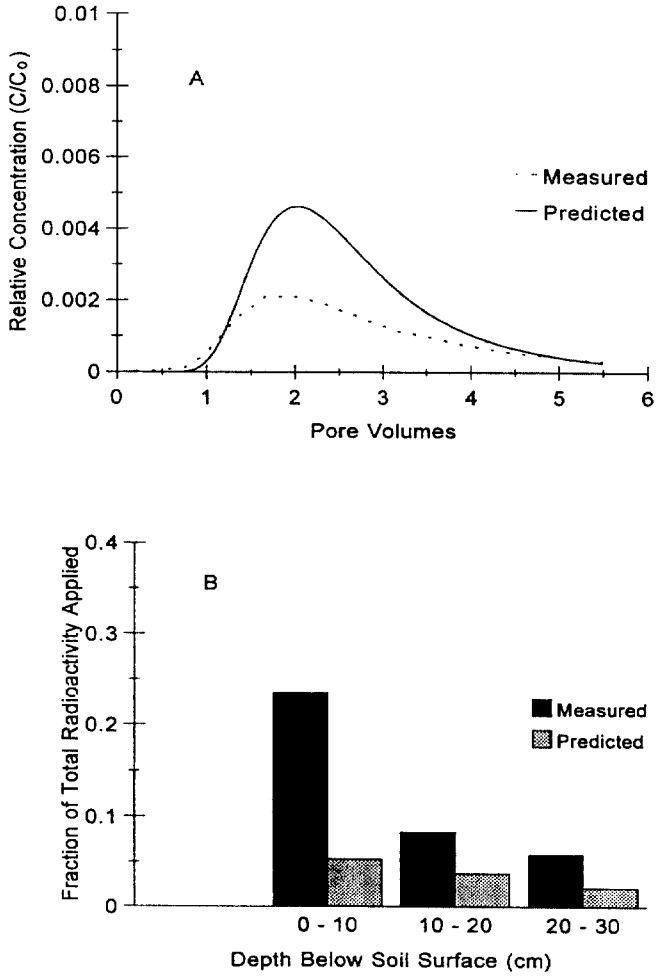


**Fig. 7. (A) Average predicted and measured effluent concentrations of acifluorfen from the intact soil columns. (B) Average predicted and measured concentrations of residual $^{14}C$ (unextractable and extractable, exclusive of acifluorfen) in three depth increments of the soil columns.**

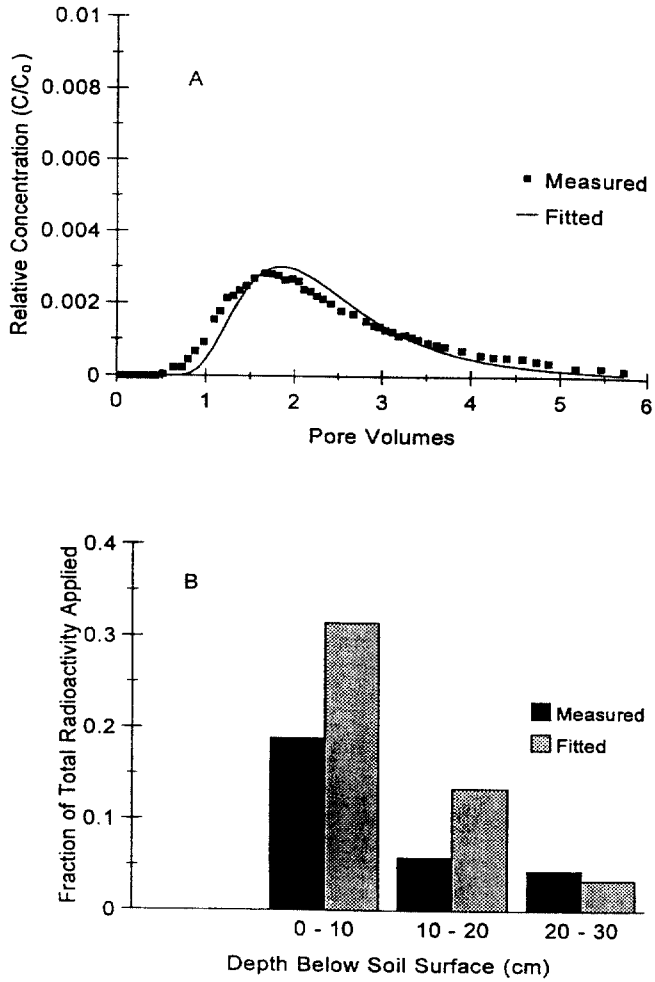


**Fig. 8. (A) Simulation of acifluorfen elution from intact and no-till (NT) soil column 1 generated using best-fit values for $k_d$ in the 0–10 and 20–30 cm depths compared with measured effluent concentrations. (B) Distribution of residual $^{14}C$ (unextractable and extractable, exclusive of acifluorfen) with depth obtained using best-fit values for $k_d$ compared with measured residual $^{14}C$.**

toward the high end of the possible range in values for the Dundee soils. On the other hand, aeration in the biometer flasks may have been artificially high with respect to field soil at similar water content (particularly below the soil surface). Thus, $k_d$s from the batch systems, though obtained at 35% moisture, may be more indicative of acifluorfen degradation under drier, more aerated conditions.

## SUMMARY

Effects of tillage on acifluorfen sorption isotherms in the Dundee CT and NT soils were related to amount and distribution of OC. Higher content of OC in the 0- to 10-cm NT led to greater acifluorfen sorption than in the surface CT soil, but more OC in the subsurface CT soils resulted in greater sorption than in the corresponding NT soils. Sorption isotherms for all six tillage–depth combinations were nonlinear. Approach to equilibrium was time-dependent and conformed to the two-site equilibrium–kinetic model. Kinetics were rapid (>70% of total sorption occurring almost instantaneously and, typically, more than 90% within 24 h) but revealed no clear trend with respect to tillage or OC content. Nevertheless, inclusion of sorption kinetics in the transport model for acifluorfen mobility gave more accurate predictions of retardation than assumption of instantaneous sorption equilibrium.

Acifluorfen degradation was more rapid in topsoil (0- to 10-cm) than in subsoil (20- to 30-cm). The first-order rate constant was larger for the CT topsoil, but there was no difference between CT and NT subsoils in rate of acifluorfen degradation. The incubation study revealed slow rates of acifluorfen mineralization and transformation to unextractable forms of $^{14}C$. There was little evidence for extractable degradation products. Analysis of effluent from the intact soil columns also indicated negligible concentration of degradation products. Thus, intermediate products of acifluorfen are apparently

highly sorbed. However, unlike the incubation study, extracts of soil column segments did contain degradation products, particularly extracts of the soil surface segments. Discrepancy between these results likely reflects greater rates of acifluorfen degradation in the intact soil columns.

Although use of batch degradation rate constants in the transport model underpredicted the extent of degradation, the model was capable of describing effluent concentration of acifluorfen and distribution of residual $^{14}C$ if degradation rate constants were optimized. Best-fit first-order rate constants were several-fold larger than rate constants calculated from the incubation study. Since acifluorfen degradation is faster under anaerobic conditions, more rapid degradation in the soil columns may have been promoted by poorer aeration than in the batch systems. Thus, degradation rate constants obtained from the mobility study may tend toward the high end of a possible range of values.

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# Anaerobic Degradation of Acifluorfen by Different Enrichment Cultures

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A laboratory study on the biodegradation of acifluorfen in anaerobic conditions was conducted. Mixed and pure cultures isolated from activated sludges of a waste water treatment plant and from a soil with a long history of acifluorfen applications could reduce acifluorfen to aminoacifluorfen in a medium with the herbicide as sole source of carbon. Addition of sodium acetate and sodium 2-nitrobenzoate to the medium enhanced and decreased the reduction rate, respectively. A further transformation of aminoacifluorfen was observed with formation of 5-[[2-chloro-4-(trifluoromethyl)phenyl]oxy]-2-aminobenzamide and 5-[[2-chloro-4-(trifluoromethyl)phenyl]oxy]-2-(acetylamino)benzoic acid.

## INTRODUCTION

Acifluorfen, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid (CAS Registry No. 50594-66-6), is a diphenyl ether herbicide largely used as sodium salt in pre-emergence control of broad-leaved weeds in soybean fields. Amino derivatives were found to be the main degradation products of several nitrodiphenyl ether herbicides in soil (Oyamada and Kuwatsuka, 1988; Niki and Kuwatsuka, 1976a). Oyamada and Kuwatsuka (1988) reported that the redox state of the soil remarkably affected the reduction of chlornitrofen: lower Eh values were associated with more rapid degradation of the herbicide. Ruzo et al. (1980) reported that photolysis of typical nitrodiphenyl ethers in solution causes reductive dehalogenation, decarboxymethylation, reduction of nitro substituents, and cleavage of the ether linkage. The degradation of diphenyl ether herbicides probably involves the action of microorganisms. Degradation of chlornitrofen was more rapid in unsterilized soils than in sterilized soils (Oyamada and Kuwatsuka, 1989). Schmidt and Braune (1987) isolated mixed bacterial populations able to degrade nitrofen within 4–5 weeks in the presence of acetate as a cosubstrate for growth. Walker et al. (1988) observed more degradation of oxyfluorfen (in water) in the presence of nonsterile sediment than with sterile sediment. In the literature, very few investigations related to the degradation of acifluorfen are reported. Draper and Casida (1983b) studied the metabolism of acifluorfen by rats and found that the predominant reaction involved the reduction of the nitro group. The disappearance of acifluorfen in five soils with different physicochemical characteristics was monitored by us (Gennari and Nègre, 1990). We found that the half-life of acifluorfen varied from 23 days to more than 112 days, depending on the soil type. Perucci and Scarponi (1993) observed a reduction of the half-life of acifluorfen in soil from 40 days to 28 days after amendment with glucose. Pusino and Gessa (1991) studied the photolysis of acifluorfen in aqueous solution and found that only decarboxylation occurred. We reported on the degradation of acifluorfen by a mixed bacterial culture in the presence of sodium 2-nitrobenzoate (Andreoni et al., 1994). The mixed culture allowed the reduction of acifluorfen to aminoacifluorfen under aerobic conditions.

The objective of this work was to determine what microbially mediated reactions acifluorfen may be subjected to under anaerobic conditions.

## MATERIALS AND METHODS

**Enrichment Cultures.** Activated sludges from a waste water treatment plant (20 mL), a soil from a field treated for 3 years with acifluorfen (5 g), or a mixed culture able to degrade acifluorfen in the presence of sodium 2-nitrobenzoate in aerobic conditions (20 mL) were used as inoculum. The inoculum was placed in 250-mL screw-cap bottles to which 20 mL of sterile water containing 2.5 mg of acifluorfen was added. Eighty milliliters of sterile mineral medium (M9) containing 0.1% sodium acetate or 0.25% sodium 2-nitrobenzoate as source of carbon and energy or without alternative carbon source was added, and the suspensions were incubated at 30 °C in the dark in an anaerobic glovebox (Anaerobic System, Forma Scientific) with $CO_2/H_2/N_2$ (10:10:80) atmosphere.

The composition of the mineral medium M9 is reported elsewhere (Gennari et al., 1991). Every 2 weeks, for 3 months, 20 mL of each culture was used to inoculate fresh herbicide medium identical to that of the parent culture. All operations were performed in an anaerobic glovebox.

**Biodegradation of Acifluorfen by Mixed Cultures.** Aliquots (20 mL each) of the enrichment cultures were placed in screw-cap bottles, and 80 mL of M9 containing acifluorfen (2.5 mg) was added with or without addition of 0.1% sodium acetate or 0.25% sodium 2-nitrobenzoate as an alternative source of carbon. All operations were performed in an anaerobic glovebox. The cultures were incubated at 30 °C in the dark in anaerobic conditions.

Nonbiological degradation of acifluorfen was assessed in sterile incubation medium. All treatments were in duplicate, and the experiment was repeated three times.

**Analysis of Acifluorfen and Aminoacifluorfen.** Five-milliliter aliquots were removed from each bottle after 0, 1, 4, 7, 14, 28, and 56 days, respectively, diluted 1:5 with acetonitrile, filtered through a 0.2-μm nylon membrane, and analyzed by HPLC. The liquid chromatograph used was a Perkin-Elmer L35 equipped with a Supelcosil $LC_{18}$ column and a diode array detector operating at 295 nm for acifluorfen analysis and at 230 nm for aminoacifluorfen analysis. The column was eluted with a mobile phase that contained 20% (v/v) water acidified to pH 3 with

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Table 1. Acifluorfen Remaining and Formation of Aminoacifluorfen in Aerobic Enrichment Cultures[a]

| | alternative carbon sources | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | acetate | | | 2-nitrobenzoate | | | none | | |
| time (days) | AC | AAC | AC + AAC | AC | AAC | AC + AAC | AC | AAC | AC + AAC |
| 0 | 100.0 | 0.0 | 100.0 | 100.0 | 0.0 | 100.0 | 100.0 | 0.0 | 100.0 |
| 1 | 101.3 | 8.9 | 110.2 | 96.3 | 1.5 | 97.8 | 101.2 | 7.8 | 109.0 |
| 4 | 98.4 | 12.2 | 110.6 | 100.7 | 2.0 | 102.7 | 100.0 | 1.2 | 101.2 |
| 7 | 94.5 | 11.7 | 106.2 | 101.1 | tr | 101.1 | 92.9 | 5.9 | 98.8 |
| 18 | 90.4 | 15.8 | 106.2 | 98.9 | 1.1 | 100.0 | 94.2 | 8.6 | 102.8 |
| 28 | 68.1 | 19.7 | 87.8 | 97.8 | 1.7 | 99.5 | 92.0 | 9.0 | 101.0 |
| 56 | 0.0 | 94.2 | 94.2 | 90.9 | tr | 90.9 | 82.5 | 8.6 | 91.1 |

[a] Data are presented as percentage of the initial molar concentration present in the cultural broth. Standard deviation <10% (mean of six replications). AC, acifluorfen; AAC, aminoacifluorfen; tr, traces.

Table 2. Acifluorfen Remaining and Formation of Aminoacifluorfen in Anaerobic Soil Enrichment Cultures[a]

| | alternative carbon sources | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | acetate | | | 2-nitrobenzoate | | | none | | |
| time (days) | AC | AAC | AC + AAC | AC | AAC | AC + AAC | AC | AAC | AC + AAC |
| 0 | 100.0 | 0.0 | 100.0 | 100.0 | 0.0 | 100.0 | 100.0 | 0.0 | 100.0 |
| 1 | 95.2 | 10.3 | 105.5 | 100.7 | 0.0 | 100.7 | 101.6 | 8.8 | 110.4 |
| 4 | 0.0 | 100.1 | 100.1 | 102.3 | 0.0 | 102.3 | 48.8 | 41.1 | 89.9 |
| 7 | 0.0 | 97.3 | 97.3 | 102.1 | 0.0 | 102.1 | 26.7 | 64.8 | 91.5 |
| 18 | 0.0 | 100.3 | 100.3 | 96.9 | 2.2 | 99.1 | 0.0 | 100.8 | 100.8 |
| 28 | 0.0 | 83.7 | 83.7 | 79.5 | tr | 79.5 | 0.0 | 82.3 | 82.3 |
| 56 | 0.0 | 80.5 | 80.5 | 86.9 | tr | 86.9 | 0.0 | 84.2 | 84.2 |

[a] Data are presented as percentage of the initial molar concentration present in the cultural broth. Standard deviation <10% (mean of six replications). AC, acifluorfen; AAC, aminoacifluorfen; tr, traces.

orthophosphoric acid and 80% (v/v) acetonitrile. The flow rate was 1 mL/min.

**Confirmation.** Identity of the compounds was confirmed by comparing the HPLC retention times and the mass spectra with those of authentic samples. Analytical grade acifluorfen and aminoacifluorfen (97% pure) were obtained from Dr. Ehrenstorfer (Augsburg, Germany).

**Isolation of Microorganisms.** Pure cultures of acifluorfen-degrading microorganisms were isolated from the mixed cultures with the dilution-plate technique. Five fast-growing cultures were selected and purified by streaking on reinforced clostridial medium (RCM, Oxoid) agar. Isolated colonies were subjected to microbiological analysis and prepared for studies of their biodegradation of acifluorfen activity.

**Biodegradation of Acifluorfen by Pure Cultures.** Washed cell inocula were suspended on M9 medium containing acifluorfen (2.5 mg) and a supplementary carbon source (0.25% sodium 2-nitrobenzoate or 0.1% sodium acetate) identical to that of the parent mixed culture. The suspensions were incubated under anaerobic conditions in the dark at 30 °C. At intervals from 0 to 56 days, 5-mL aliquots of the cultural broth were removed for the determination of acifluorfen and aminoacifluorfen residual concentration following the techniques described above.

**Extraction of Metabolites.** At the end of each degradation experiment (after 56 days of incubation) extraction and determination of metabolites were attempted. The content of each bottle was transferred to a 250-mL separatory funnel and extracted two times with 50 mL of dichloromethane and two times with ether. The aqueous phase was successively acidified to pH 2 with 1 N hydrochloric acid and extracted two times with 50 mL of dichloromethane and two times with 50 mL of ether. The organic phases were collected together, dried over anhydrous sodium sulfate, evaporated to dryness, resuspended in 1 mL of acetone, layered onto 0.25-mm $C_{18}$ thin-layer chromatography (TLC) plates, and developed with a methanol/toluene 1:8 (v/v) solvent system. Separate bands of metabolites, detected under UV lamps, were scraped off and eluted from the $C_{18}$ stationary phase and 1 mL of methanol; then they were analyzed by HPLC and mass spectrometry.

**Identification of Metabolites.** HPLC analyses were performed under the same analytical conditions used for the determination of acifluorfen and aminoacifluorfen.

Mass spectrometric experiments were run on a Finnigan-MAT 95 Q instrument with magnetic, electrostatic, and quadrupole analyzers mounted in series. Desorption chemical ionization (DCI) (Baldwin and McLafferty, 1973; Cotter, 1980; Vincenti et al., 1992) analyses were executed by loading 1 μL of methanol solution of analytes onto the DCI rhenium filament. The filament was subsequently introduced into the ion source through a probe and heated by an electric current at a heating rate of 4000 °C/min. Methane (0.5 mbar) was used as the reagent gas. Both positive and negative ion spectra were recorded yielding, respectively, $[MH]^+$ and $[M]^-$ molecular ions of the analytes. Ion source temperature was maintained low (50 °C); the electron energy was set to 200 eV, the emission current set to 0.2 mA, and the magnetic analyzer scanned from $m/z$ 130 to 750 at 0.8 s/decade. Electron impact mass spectra were recorded using the same probe utilized in DCI. The electron energy was set to 70 eV, the emission current set to 1 mA, and the magnetic analyzer scanned from $m/z$ 35 to 450 at 0.8 s/decade.

## RESULTS

**Biodegradation of Acifluorfen by Mixed Cultures.** The results of the experiments, regarding the disappearance of acifluorfen and the evolution of aminoacifluorfen with time, are reported in Tables 1–3. In all conditions studied, breakdown of the acifluorfen molecule was accompanied by the appearance of aminoacifluorfen. The mixed cultures derived both from soil previously treated with acifluorfen and from activated sludges demonstrated higher capacity to reduce acifluorfen than the mixed culture previously enriched in aerobic conditions. Almost quantitative conversion of acifluorfen to aminoacifluorfen is observed when the medium contains the herbicide as sole source of carbon. In these cultural conditions a limited growth of the microbial populations was observed, although the degradation proceeds via a reductive pathway not yielding carbon or energy. This growth might be initially attributed to the uncharacterized dissolved organic carbon in the inocula and later to the organic carbon deriving from dead cells present in the preculture. The conversion of acifluorfen to aminoacifluorfen occurs within the first 18 and 28 days in the bottles containing mixed cultures from soil and activated sludges, respectively. In the bottles with the aerobic mixed culture only 17.5% of acifluorfen

Table 3. Acifluorfen Remaining and Formation of Aminoacifluorfen in Anaerobic Activated Sludge Enrichment Cultures[a]

| | alternative carbon sources | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | acetate | | | 2-nitrobenzoate | | | none | | |
| time (days) | AC | AAC | AC + AAC | AC | AAC | AC + AAC | AC | AAC | AC + AAC |
| 0 | 100.0 | 0.0 | 100.0 | 100.0 | 0.0 | 100.0 | 100.0 | 0.0 | 100.0 |
| 1 | 99.8 | 11.1 | 110.9 | 101.0 | 0.0 | 101.0 | 95.7 | 7.8 | 103.5 |
| 4 | 69.1 | 25.8 | 94.9 | 99.1 | 1.1 | 100.2 | 95.2 | 15.3 | 110.5 |
| 7 | 49.3 | 43.6 | 92.9 | 73.3 | 16.3 | 89.6 | 72.1 | 27.5 | 99.6 |
| 18 | 0.0 | 101.3 | 101.3 | 23.2 | 57.1 | 80.3 | 12.3 | 90.9 | 103.2 |
| 28 | 0.0 | 96.9 | 96.9 | 2.0 | 72.3 | 74.3 | 0.0 | 100.0 | 100.0 |
| 56 | 0.0 | 92.2 | 92.2 | 0.0 | 45.6 | 45.6 | 0.0 | 91.5 | 91.5 |

[a] Data are presented as percentage of the initial molar concentration present in the cultural broth. Standard deviation <10% (mean of six replications). AC, acifluorfen; AAC, aminoacifluorfen.

was reduced after an incubation period of 56 days. The disappearance of acifluorfen seems to be affected by the addition of alternative carbon sources. In all cases studied, the addition of sodium acetate in the medium enhanced the reduction rate of acifluorfen to aminoacifluorfen, whereas the addition of sodium 2-nitrobenzoate reduced the conversion rate. In the presence of sodium 2-nitrobenzoate, 100% of the acifluorfen was degraded after 56 days of incubation when mixed cultures derived from activated sludges were utilized, whereas the degradation was negligible with the other mixed cultures.

It is worth noting that the increase of aminoacifluorfen concentration is proportional to the decrease of acifluorfen in the first 18-28 days, depending on the experiments. Subsequently, the mass balance based on these two components decreases, indicating the degradation of the first metabolite. The disappearance of aminoacifluorfen was highest in the mixed culture derived from activated sludges containing sodium 2-nitrobenzoate (Table 3). However, no further metabolites were detected by direct HPLC analysis of the cultural broth. To identify the compounds derived from the biotransformation of the aminoacifluorfen primary metabolite, an extraction from whole culture broths after 56 days of incubation was made, as described under Materials and Methods.

No degradation of acifluorfen and aminoacifluorfen was detected in bottles incubated under sterile conditions, confirming that the microorganisms are responsible for degradation of these compounds.

**Identity of Metabolites of Aminoacifluorfen.** TLC analysis of the extracts revealed the presence of four bands, corresponding to $R_f$ 0.66, 0.77, 0.81, and 0.87 which were removed, extracted with methanol, and analyzed by HPLC. The compounds eluting at $R_f$ 0.66 and 0.87 turned out to be acifluorfen and aminoacifluorfen, respectively. Compounds at $R_f$ 0.77 and 0.81, eluting from the HPLC column after 3.82 and 3.79 min, were named metabolites II and III and were identified by means of mass spectrometric techniques.

Positive and negative DCI mass spectra (not reported here) were exploited to determine unequivocably the molecular weight of metabolites. Positive ion DCI mass spectra exhibited, for each metabolite, the protonated molecular ion $[MH]^+$ as the base peak, together with a fragment $[M-F]^-$. Negative ion DCI mass spectra showed the occurrence of the molecular ion $[M]^-$, together with an abundant fragment $[M - HCl]^-$. From these data it was possible to establish nominal molecular weights of 331, 330, and 373 for aminoacifluorfen, metabolite II, and metabolite III, respectively.

D-EI mass spectra for aminoacifluorfen, metabolite II and metabolite III are reported in Figure 1. The spectrum relative to aminoacifluorfen (Figure 1a) exhibits the molecular ion as the base peak and some meaningful

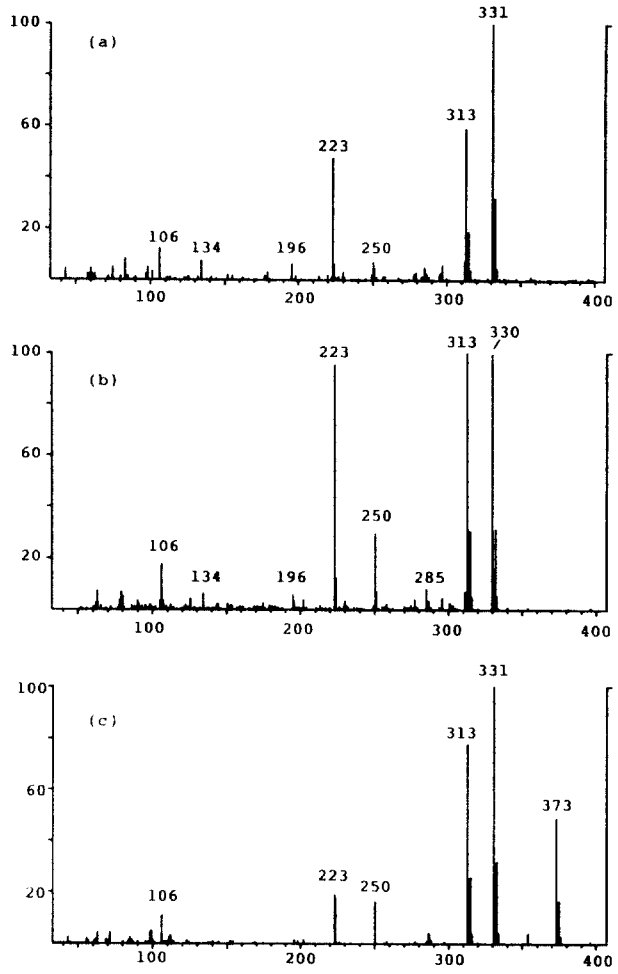


Figure 1. Direct electron impact mass spectra of metabolites of acifluorfen, extracted from a preparative TLC plate: (a) aminoacifluorfen; (b) metabolite II; (c) metabolite III.

fragments, such as $m/z$ 313 $[M - H_2O]^+$ and $m/z$ 250 $[M - HCl - COOH]^+$. $m/z$ 223 might arise from further HCN loss from $m/z$ 250. Lastly, fragment ions at $m/z$ 196 and 179 are relative to the halogenated benzene ring, with and without an OH group, respectively, while fragments at $m/z$ 134 and 106 are relative to the other aromatic ring. This spectrum, obtained from a TLC extract and representing an acifluorfen metabolite, was compared with that obtained from an authentic aminoacifluorfen standard and turned out to be identical.

For the other two metabolites, authentic standards are not available nor are the spectra included in any general and specific mass spectral library. The identification of their structures is based on the interpretation of mass spectra and should be regarded as tentative. The spectrum of metabolite II (Figure 1b) is similar to that of ami

Table 4. Acifluorfen Remaining and Formation of Aminoacifluorfen in Anaerobic Pure Cultures[a]

| | pure cultures | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C1 | | | C2 | | | C3 | | | C4 | | |
| time (days) | AC | AAC | AC + AAC | AC | AAC | AC + AAC | AC | AAC | AC + AAC | AC | AAC | AC + AAC |
| 0 | 100.0 | 0.0 | 100.0 | 100.0 | 0.0 | 100.0 | 100.0 | 0.0 | 100.0 | 100.0 | 0.0 | 100.0 |
| 7 | 11.3 | 63.5 | 74.8 | 101.0 | 0.0 | 101.0 | 99.0 | 0.0 | 99.0 | 100.0 | 0.0 | 100.0 |
| 14 | 11.3 | 46.5 | 57.8 | 95.4 | 0.0 | 95.4 | 103.3 | 0.0 | 103.3 | 104.0 | 0.0 | 104.0 |
| 28 | 10.1 | 52.5 | 62.6 | 74.4 | 6.7 | 81.1 | 41.1 | 5.9 | 47.0 | 41.6 | 20.8 | 62.4 |
| 56 | 10.1 | 37.8 | 47.9 | 71.8 | 6.9 | 78.7 | 41.1 | 23.4 | 64.5 | 40.6 | 0.0 | 40.6 |

[a] Data are presented as percentage of the initial molar concentration present in the cultural broth. Standard deviation <10% (mean of four replications). AC, acifluorfen; AAC, aminoacifluorfen.

Table 5. Morphological and Physiological Characteristics of the Pure Cultures Able To Metabolize Acifluorfen

| isolate | Gram reaction | shape and dimension | catalase | optimum growth conditions | | sporulation characteristics | parasporal crystals |
|---|---|---|---|---|---|---|---|
| | | | | anaerobic | aerobic | | |
| C1 | positive | rods, single motile | positive | LB agar[a] at 37 °C | Nutr agar (Difco) at 37 °C | spore cylindrical subterminal or central | present |
| C2 | positive | rods, single irregular | negative | PYG agar[b] at 30 °C | PYG agar at 30 °C | not detected | not detected |
| C3 | positive | rods, single or short chains | negative | BHI broth[c] at 37 °C | BHI broth at 37 °C | not detected | not detected |
| C4 | negative | rods, motile very small | negative | RCM (Oxoid) at 30 °C | no growth | not detected | not detected |

[a] LB agar: triptone 1%; yeast extract 0.5%; NaCl 0.5%; agar 1.2%; pH 7.5. [b] PYG agar: glucose 2%; yeast extract 0.5%; peptone 0.5%; agar 1%; $CaCO_3$ spread on the surface. [c] BHI suppl. agar = BHI (Difco) 3.7%; yeast extract 0.5%; cysteine-HCl-$H_2O$ 0.05%; pH 7.2; supplemented with emine 0.5 mg/100 mL and K vitamin.

noacifluorfen, except for the molecular ion, which is shifted 1 mass unit downward. This suggests that the carboxylic functional group of aminoacifluorfen has been modified, in metabolite II, to an amidic group, which readily fragments by releasing an $NH_3$ molecule (instead of a $H_2O$ molecule) to generate the ion $m/z$ 313, identical to that produced from aminoacifluorfen. The rest of the fragment ions arise from $m/z$ 313 by consecutive bond cleavages and are identical in the two spectra, because the parent ions have the same structure.

The mass spectrum of metabolite III is again similar to that of aminoacifluorfen, but the molecular ion is 42 mass units higher. A neutral loss of 42 Da and an abundant $m/z$ 43 ion indicate the probable presence of an acetyl group in the structure. Also in this case, the acetyl group is easily removed as a carbene ($CH_2$=CO) neutral loss, releasing a hydrogen on the NH group. Thus, a fragment ion identical to the molecular ion of aminoacifluorfen is created, which fragments further by similar pathways.

**Biodegradation of Acifluorfen by Isolated Microorganisms.** No anaerobic organism was isolated from the cultural broths containing the aerobic mixed culture in any of the three cultural conditions tested, probably because this population does not grow in anaerobic conditions. Negligible turbidity of the cultural broth was observed even when a supplementary carbon source was added to the medium, demonstrating a very low growth of this microbial population in the anaerobic biodegradation experiments. Three different organisms were isolated from the mixed cultures arising from activated sludges (C1, C2, and C3) and two from soil (C4 and C5). When their ability to degrade acifluorfen was tested, disappearance of the herbicide was observed with four of these organisms (Table 4). However, in these experiments conducted with individual species, the degradation of acifluorfen was never accompanied by a proportional formation of aminoacifluorfen. This might indicate that the isolated populations are able to metabolize aminoacifluorfen more quickly than the parent mixed cultures. Also in these cases, no metabolite of aminoacifluorfen was detected by direct HPLC analysis of the cultural broths. Metabolites II and III were again detected upon extraction from the cultural broths.

Morphological and physiological characteristics of the four microorganisms capable of degrading acifluorfen are given in Table 5.

## DISCUSSION

Both mixed and pure bacterial cultures were identified which could degrade acifluorfen in anaerobic conditions. The microbial populations carried out the reduction of the nitro group of acifluorfen in the mineral medium with or without an additional carbon source. Reduction was enhanced when sodium acetate was added to the medium, whereas it was inhibited when sodium 2-nitrobenzoate was added. Our previous studies showed that reduction of acifluorfen by microorganisms may also occur in aerobic conditions (Andreoni et al., 1994).

Formation of the amino derivative of nitrodiphenyl ethers has been observed in other studies. Draper and Casida (1983a,b) observed metabolic reduction of several nitrodiphenyl ether herbicides to amino derivatives by rats presumably via nitroso and the highly instable hydroxylamine intermediates. While studying the degradation of chlornitrofen, nitrofen, and chlomethoxynil in soil under flooded and upland conditions, Niki and Kuwatsuka (1976a) observed formation of the amino derivatives in flooded conditions but not under upland conditions. These authors also observed disappearance of the amino derivatives, but they were not able to assess whether the metabolite was degraded or adsorbed on the soil constituents. Oyamada and Kuwatsuka (1989) reported the reduction of the herbicide chlornitrofen in soil under flooded conditions. They found that the herbicides was reduced by $Fe^{2+}$, but they also demonstrated that the $Fe^{2+}$ content in the soil depended on the microbial activity. In our experimental conditions 1.2 ppm of $FeSO_4 \cdot 7H_2O$ was present in the medium for microbial growth, but reduction of acifluorfen to aminoacifluorfen was observed only in the presence of microorganisms, indicating the importance of the microflora in this type of reaction.

The reduction of the nitro group of acifluorfen is not a major detoxification step in plants. Frear et al. (1983), studying the metabolism of acifluorfen in soybean, observed cleavage of the diphenyl ether bond and formation of conjugates with homoglutathione, cysteine, and glucose.

Further metabolism of aminoacifluorfen was observed with formation of two metabolites, namely 5-[[2-chloro-

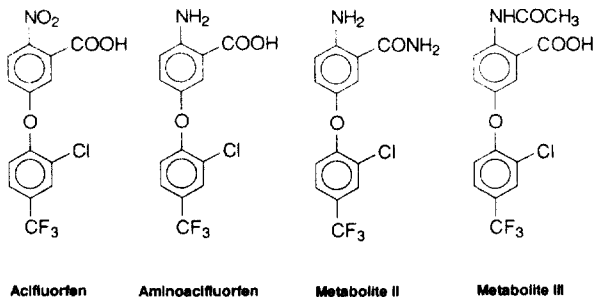


**Figure 2.** Structures of acifluorfen and its metabolites.

4-(trifluoromethyl)phenyl]oxy]-2-aminobenzamide (metabolite II) and 5-[[2-chloro-4-(trifluoromethyl)phenyl]-oxy]-2-(acetylamino)benzoic acid (metabolite III). The structures of acifluorfen and its metabolites are given in Figure 2.

Acylation of aromatic amines has frequently been observed, and it is regarded as a detoxification process used by soil micoorganisms, higher plants, and animals (Tweedy et al., 1970; Bollag et al., 1978; Parris, 1980). The acylation of chlomethoxynil in soil has been reported by Niki and Kuwatsuka (1976b). They also found trace amounts of 2,4-dichlorophenol and its dechlorinated derivative. In our study neither cleavage of the ether bond nor reductive dechlorination was observed. Incorporation of an amino group was observed in our study (metabolite II). Some microorganisms are known to add nitrate to aromatic compounds (Sylvestre et al., 1982), but no evidence has been found in the literature on biotransformations involving conversion of carboxylic to amidic groups.

From this study, it appears that reduction and conjugation of acifluorfen occur but these processes result in only a minor modification of the molecule and not its complete decomposition. This observation has significance as to the transport and fate of acifluorfen in soil, since the long-lived products of transformation might be adsorbed on the surface of colloidal soil particles or leached through the subsurface to groundwater. On the other hand, the amino derivative of acifluorfen was reported to be non-mutagenic against *Salmonella typhimurium* (strain TA 100) even after metabolic activation (+S9) (Draper and Casida, 1983b).

Further studies are in progress to assess the capability of mixed and pure cultures to degrade acifluorfen more extensively under different cultural conditions.

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# Effect of Soil Properties on Adsorption and Desorption of Acifluorfen

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The adsorption and subsequent desorption of acifluorfen were investigated in five soils of various physical and chemical properties. The adsorption and desorption of acifluorfen were essentially dependent upon pH, organic carbon content, and ammonium oxalate extractable iron content of the soil but not on clay content. The data for the adsorption experiments fit the Freundlich equation and, depending on the soil, the adsorption constant $K_f$ ranged from 0.57 to 43.10, while $n$ varied from 0.84 to 1.04. Removal of organic matter from soil with $H_2O_2$ caused loss of the capacity to adsorb acifluorfen. Desorption of acifluorfen showed a hysteresis effect.

**Keywords:** *Acifluorfen; soil; adsorption; desorption*

## INTRODUCTION

Acifluorfen, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid, is a highly effective postemergence herbicide used in the selective control of broad-leaf weeds in soybeans (Johnson et al., 1978; Wills and McWhorter, 1981). Acifluorfen contains a carboxylic acid group whose p$K_a$ is 3.5 (Roy et al., 1983). It is expected that acifluorfen exists as the dissociated anion in most agricultural soils since the pH of these soils usually exceeds the p$K_a$ of the acid. Negatively charged adsorption sites were therefore not expected to contribute to the adsorption process. Recent studies on the interaction of sodium acifluorfen with montmorillonite saturated with Cu(II), Ca(II), Al(III), and Fe(III) showed that the herbicide was not adsorbed on the clays but formed carboxylate complexes with the exchangeable cations which precipitate on the outer surfaces of the clays (Pusino et al., 1991). The literature indicates that adsorption of acidic pesticides is positively related to the organic carbon content and pH of the soils (O'Connor and Anderson, 1974; Farmer and Aochi, 1974; Murray and Hall, 1989). Ruggiero et al. (1992) reported that acifluorfen was adsorbed on a humic acid extracted and purified from an Andosol soil and that adsorption was partially reversible. Since information on the behavior of acifluorfen in soil is essential in predicting its leaching potential and contamination of groundwater, we conducted a study on the adsorption and desorption of this herbicide in five soils of various physical and chemical properties.

## MATERIALS AND METHODS

**Adsorption.** Five soils differing widely in their physicochemical characteristics (Table 1) were used in this study. Soils were collected at 0–15 cm depth, air-dried, and passed through a 2 mm sieve. Herbicide solutions were made in 0.01 N $CaCl_2$ and contained 1, 2, 4, 8, 12, 16, or 20 mg $L^{-1}$ acifluorfen. Precipitation of acifluorfen in the presence of calcium is excluded as the ionic product of the two species in solution was below the solubility product ($1.5 \times 10^{-9}$) of the complex Ca(acifluorfen)$_2$ (A. Pusino, personal communication). Adsorption of acifluorfen was studied using a batch equilibrium method. Duplicate samples of 5 g of air-dried soil and 25 mL of the herbicide solution were placed in 50 mL of polyethylene bottles (PE). Additionally, two samples for each concentration without soil were carried through the procedure to account for possible losses due to adsorption on PE bottles, volatilization, or degradation. The samples were equilibrated by shaking on a reciprocating mechanical shaker for 16 h at 25 °C. A previous study (Gennari and Nègre, 1990) indicated no significant change in acifluorfen adsorption between 2 and 48 h of shaking at 25 °C for soils A–D and between 16 and 48 h of shaking for soil E. Following equilibration, the suspensions were centrifuged for 15 min at 1200$g$ and the supernatant was filtered on a Whatman No. 42 paper filter. The amount of acifluorfen in supernatant was determined by HPLC using a Varian 5020 instrument equipped with a UV–vis detector operating at 296 nm and a 250 × 4 mm LiChrospher RP 18 analytical column. The mobile phase (1 mL $min^{-1}$) was composed of water acidified to pH 3 with orthophosphoric acid plus acetonitrile (30:70 v:v) (Gennari et al., 1990). The method limit of detection was 6 ppb. The amounts of acifluorfen retained by the soils were calculated by the formula

$$x/m = (C_0 - C)v/w$$

where $x/m$ is the concentration in soil (mg $kg^{-1}$), $C_0$ is the initial concentration in solution (mg $L^{-1}$), $C$ is the final concentration in solution (mg $L^{-1}$), $v$ is the solution volume (25 mL), and $w$ is the weight of soil (5 g).

**Desorption.** The same sample bottles used for the adsorption studies were used for determining successive desorption of acifluorfen from the soils. Once the adsorption equilibrium had been reached, the suspension was centrifuged for 15 min at 1200$g$, and then 15 mL of supernatant was replaced with

**Table 1. Selected Properties of the Soils**

| | particle size distribution, % | | | | |
|---|---|---|---|---|---|
| soil[a] | coarse sand, 2–0.2 | fine sand, 0.2–0.05 | coarse silt, 0.05–0.02 | fine silt, 0.02–0.002 | clay, <0.002 |
| A | 0.9 | 9.5 | 9.8 | 36.4 | 43.4 |
| B | 2.7 | 31.6 | 18.1 | 22.7 | 24.9 |
| C | 34.4 | 38.2 | 15.4 | 9.8 | 2.2 |
| D | 54.1 | 13.4 | 15.2 | 11.5 | 2.9 |
| E | 28.4 | 16.4 | 7.5 | 22.1 | 25.7 |

| | oxalate extractable, g/100 g of soil | | | | |
|---|---|---|---|---|---|
| soil | Fe | Al | CEC, mequiv/100 g | OC, % | pH 1:2.5 soil:water |
| A | 0.3 | 0.1 | 20.9 | 0.98 | 8.0 |
| B | 0.3 | 0.1 | 13.9 | 0.83 | 7.9 |
| C | 0.2 | 0.2 | 11.6 | 2.35 | 5.6 |
| D | 0.6 | 1.2 | 54.2 | 11.31 | 5.6 |
| E | 1.5 | 0.5 | 56.1 | 14.80 | 4.6 |

[a] A and B, Entisol; C, Inceptisol; D, Andosol; E, Istosol.

**Table 2. Freundlich Constants and Coefficient of Determination ($r^2$) for Acifluorfen Adsorption and Desorption**

| soil | isotherm | initial concn, μg/mL | $K_f$ | $n$ | $r^2$ [a] | $K^b$ | $K_{OC}$ [c] | $n_{ads}/n_{des}$ |
|---|---|---|---|---|---|---|---|---|
| A | adsorption | | 0.64 | 1.05 | 0.964*** | | 65.3 | |
| | desorption | 20.0 | 7.38 | 0.03 | 0.997* | 1053.1 | | 35.1 |
| | desorption | 8.0 | 0.92 | 0.45 | 0.999* | 43.8 | | 2.3 |
| B | adsorption | | 0.57 | 1.01 | 0.965** | | 68.7 | |
| | desorption | 20.0 | 6.94 | 0.09 | 0.892* | 1117.5 | | 11.1 |
| | desorption | 8.0 | 2.23 | 0.16 | 0.981* | 291.2 | | 6.3 |
| C | adsorption | | 5.48 | 1.04 | 0.985*** | | 233.2 | |
| | desorption | 20.0 | 19.06 | 0.45 | 0.991*** | 247.3 | | 2.3 |
| | desorption | 8.0 | 13.51 | 0.46 | 0.983*** | 146.4 | | 2.2 |
| D | adsorption | | 16.22 | 0.84 | 0.996*** | | 143.5 | |
| | desorption | 20.0 | 25.17 | 0.56 | 0.998*** | 55.4 | | 1.5 |
| | desorption | 8.0 | 21.07 | 0.52 | 0.996*** | 30.1 | | 1.6 |
| E | adsorption | | 43.11 | 0.86 | 0.997*** | | 291.3 | |
| | desorption | 20.0 | 62.34 | 0.42 | 0.983*** | 44.6 | | 2.2 |
| | desorption | 8.0 | 37.06 | 0.35 | 0.956*** | −0.1 | | 2.5 |
| Eoss[d] | adsorption | | 21.75 | 0.88 | 0.999*** | | 560.6 | |
| | desorption | 20.0 | 18.89 | 0.92 | 0.954*** | −0.1 | | 0.9 |
| | desorption | 8.0 | 18.54 | 0.96 | 0.941*** | −0.1 | | 0.9 |

[a] ***, $P < 0.001$; **, $0.001 < P < 0.01$; *, $0.01 < P < 0.05$. [b] $K = (K_{ides} - k_{tad}/K_{fads} \times 100$. [c] $K_{OC} = (K_F \times 100)/\%$ OC. [d] Eoss, soil E treated with 400 mL of $H_2O_2$.

15 mL of 0.01 N $CaCl_2$. The bottles were manually shaken to disperse the soil pellets and then mechanically shaken for 2 h and recentrifuged. This process was repeated four times. Preliminary trials showed that the variation in desorption of acifluorfen by the soil between 2, 16, and 24 h of equilibrium period was within the variability of the analytical method (2%). This result allows us to think that desorption equilibrium was reached within 2 h. The concentration of acifluorfen present in the supernatant after each desorption step was assayed by HPLC, and the amount of acifluorfen adsorbed onto the soil after each desorption process was calculated by difference. The adsorption and desorption isotherms were obtained by plotting the concentration of acifluorfen in soil (milligrams per kilogram) versus the concentration of acifluorfen in solution at equilibrium (milligrams per liter). To understand better the role of organic matter on the adsorption–desorption process of acifluorfen, isotherms were also determined with soil E pretreated with $H_2O_2$ to oxidize organic matter. A 100 g sample of soil E was treated with about 400 mL of $H_2O_2$ to obtain partial oxidation of organic matter. Another 100 g sample was treated with $H_2O_2$ until the maximum removal of organic matter which can be obtained with this method was reached. The amounts of organic carbon in soil E after treatments with $H_2O_2$ were 3.88% and 0.55%, respectively.

## RESULTS AND DISCUSSION

**Adsorption.** All of the adsorption data were described by the empirical Freundlich equation

$$\log x/m = \log K_f + n \log C$$

where $x/m$ is the concentration of the adsorbate per unit amount of adsorbant (mg kg$^{-1}$), $C$ is the adsorbate concentration in solution after equilibration (mg L$^{-1}$), and $K_f$ (mg$^{1-n}$ kg$^{-1}$ L$^n$) and $n$ are constants relative to the affinity of the adsorbent for the adsorbate and to the degree of curvative of the isotherm, respectively.

Freundlich constants $K_f$ and $n$ and the corresponding coefficient of determination ($r^2$) for the relationship between log $n$ and log $C$ are given in Table 2. In general, the isotherms fit the Freundlich adsorption equation with $r^2$ values >0.964. The adsorption isotherms of acifluorfen by the soils A–C, with organic carbon content equal to or lower than 2.4%, are of C type according to the classification of Giles et al. (1960). This indicates that the amount of acifluorfen adsorbed onto the soils was independent of the initial concentration of the acifluorfen solution. The soils with a higher organic carbon content gave isotherms of the L type, which indicates that there is a great affinity of these soils for the pesticide at low pesticide concentrations.

The value of $K_f$ for the five soils varied from 0.57 for soil B to 43.10 for soil E. The $K_f$ values, when subjected to simple correlation analysis with soils properties, yielded significant correlation with [H$^+$] ($r^2 = 0.92$; $P = 0.04$), organic carbon ($r^2 = 0.93$; $P = 0.03$), and ammonium oxalate extractable iron content ($r^2 = 0.97$; $P = 0.01$). No significant correlations were found between $K_f$ values and cation exchange capacity, ammonium oxalate Al content, or percent of sand, clay, or silt.

Since the adsorption of acifluorfen was highly correlated with changes in the organic carbon content, reduction or removal of organic matter was expected to reduce greatly the adsorptive capacity of the soils for the herbicide. Reduction of organic carbon in soil E from 14.8% to 3.8% decreased the adsorptive affinity of soil E for the pesticide. As can be seen in Table 2, the $K_f$ value decreased from 43.10 to 21.75. However, no variation of $n$ value occurred. Referring $K_f$ values to the organic carbon content of the soil, we observed a higher value for oxidized than for untreated soil E (Table 2). One possible explanation is that the treatment with hydrogen peroxide induced a selective disruption of the organic matter. Reduction of organic matter in soil E to 0.55% resulted in a negligible ($x/m$ < 0.3 mg kg$^{-1}$) adsorption, and no significant relationship between adsorption and herbicide concentration was found. The decrease in acifluorfen adsorptive capacity of soil E following oxidation confirms that acifluorfen has a high affinity for the organic adsorptive surfaces. This supports what many workers have reported in the literature about the affinity of anionic pesticides for the organic matter of the soil (O'Connor and Anderson, 1974; Reddy and Gambrell, 1987; Senesi and Chen, 1989).

The significant correlation between acifluorfen adsorption coefficient $K_f$ and ammonium oxalate extractable iron indicates the importance of amorphous and less crystalline iron oxides in acifluorfen adsorption in soils. Interaction between oxides and hydrous oxides

of iron and anionic organic compounds is well documented (Watson et al., 1973; Huang et al., 1977; McConnel and Hossner, 1985; Thoisy-Dur et al., 1988). The adsorption of carboxylic acids on iron oxide surfaces is essentially attributed to a ligand exchange mechanism involving the OH or $OH_2$ groups on the iron oxide surface and the carboxylic group of the organic compound (Kung and McBride, 1989). The pH is indicated to be an important factor in adsorption of anionic pesticides on iron oxides, with decreasing pH favoring increased adsorption (Thoisy-Dur et al., 1988). The increase of adsorption with the decrease in pH is generally assumed to be due to the increase in net positive charge at the surface of the iron oxides. The adsorption is characterized by an envelope showing a maximum adsorption at a pH near the $pK_a$ of the pesticide when the increase of the surface positive charge is counterbalanced by a decrease of the anionic form of the adsorbate (Schwertmann et al., 1986).

Pusino et al. (1993) reported a decrease of adsorption of acifluorfen with increasing pH. Also in the present study an inverse relationship was found between soil $[H^+]$ and adsorption of acifluorfen. The pH of the soils varied between 4.8 and 8.5. Since the $pK_a$ of acifluorfen is 3.5, the herbicide can be expected to be always in anionic form. Since the zero point charge of pure amorphous iron oxide occurs at approximately pH 8.5 (Sposito, 1989), it is possible that an interaction takes place between the positively charged amorphous iron surface at pH below 8.5 and the negatively charged acifluorfen.

**Desorption.** By fitting desorption data to the linearized log-transformed Freundlich equation, we obtained the $K_f$ values ($K_{fdes}$) and $n$ values listed in Table 2. The desorption isotherms of acifluorfen had $n$ values lower compared to the adsorption isotherms, indicating an hysteretic desorption process. The values for the ratio $n_{ads}/n_{des}$ are reported in Table 2. These values ranged between 1.5 and 2.5 for soils C–E, whereas they were much higher in soils A and B. Values of $n_{ads}/n_{des}$ close to 2.3 were reported in several studies on adsorption and desorption of pesticides (Hornsby and Davidson, 1973; Swanson and Dutt, 1973; Van Genuchten et al., 1977). The foregoing results indicate that soils A and B, with the lowest adsorption capacity, show also the least tendency to desorb acifluorfen. However, the irreversibility of the adsorption process in these soils was lower at low adsorption levels. This is confirmed by the $K$ values (the percent change in the adsorption–desorption $K_f$ value) for soils A and B compared with that for soils C–E (Table 2).

Coincidence of the desorption–adsorption isotherms was found for soil E partially oxidized with hydrogen peroxide, indicating a complete reversibility of the adsorption process in this soil. The reversibility of the adsorption process in the oxidized soil compared with the partial irreversibility in the natural ones suggests that the nature of the organic matter may also play a role in the adsorption process. This statement is supported by several works (Calvet, 1989; Chassin and Calvet, 1984; Gerstl and Kliger, 1990) which reported that the magnitude and the mechanism of adsorption of several pesticides in soil and sediments can be related to the nature of the organic matter. This fact would also offer a key as to why acifluorfen was poorly adsorbed by soils A and B (with low organic matter content) but the process was more irreversible than in the other soils.

Several hypotheses to explain herbicide hysteresis have been proposed, including nonattainment of equilibrium during desorption or loss of pesticide by volatilization and chemical or biological degradation (Koskinen and Cheng, 1983; Koskinen et al., 1979). The possibility that hysteresis in the desorption of acifluorfen was due to nonattainment of equilibrium was investigated by varying the equilibration time. Increasing the equilibration time from 2 to 24 h did not affect the amount of observed hysteresis. These data indicate that hysteresis was not the result of inadequate equilibration. The possibility of loss of pesticide by volatilization or degradation can also be ruled out as increasing times of desorption did not cause hysteresis increase. Moreover, no detectable loss of acifluorfen was shown from analyses of blank samples.

In conclusion, though the cause of observed hysteresis is still unknown, it seems reasonable to assume that it could be the result of irreversible binding of acifluorfen to the soil. Further studies would be necessary to determine the binding mechanisms that might be responsible for irreversible adsorption.

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# Acifluorfen Sorption and Sorption Kinetics in Soil

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Factors which influence kinetics of sorption for acifluorfen, a postemergence herbicide, in soil were evaluated. Twelve soils of varying characteristics were used in this study. Sorption kinetics experiments with 5.86 $\mu$M $^{14}$C-labeled (19.6 Bq mL$^{-1}$) acifluorfen were conducted for equilibration times up to 96 h. Sorption was rapid for all soils, and most soils reached a pseudo-equilibrium after 24−48 h of equilibration. Apparent sorption increased at later times, possibly reflecting sorption of acifluorfen or metabolites, since acifluorfen is subject to biotransformation. Further investigation comparing sorption at 4 and 25 °C indicated that microbial metabolism was occurring for some soils at equilibration times longer than 24−48 h. Aminoacifluorfen was observed in methanol extracts of soils equilibrated 96 h. Soils were equilibrated for 24 h with $^{14}$C-labeled acifluorfen (2.6−65.8 $\mu$M, 19.5 Bq mL$^{-1}$) to obtain sorption isotherms. Capacity for acifluorfen sorption in these soils generally increased with increasing soil organic carbon content, cation exchange capacity, and soil acidity.

**Keywords:** *Herbicide; acifluorfen; diphenyl ether; sorption; organic carbon; tillage*

## INTRODUCTION

Acifluorfen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid) is used extensively as a postemergence herbicide in soybeans (*Glycine max* L.) (Johnson et al., 1978) and peanuts (*Arachis hypogaea*). Although postemergence herbicides are applied to foliage (Ritter and Coble, 1981a,b; Hook and Glenn, 1984; Willingham et al., 1989), some herbicide likely reaches the soil surface, depending on canopy density and washoff (Reddy et al., 1994).

Upon contact with soil, acifluorfen is subject to sorption processes. Mechanisms of herbicide sorption to organic components in soil include hydrogen bonding, electrostatic interaction, van der Waals forces, and hydrophobic partitioning (Hasset and Banwart, 1989; Stevenson, 1976). Acifluorfen sorption in soil has correlated positively with soil organic matter content (Pusino et al., 1993; Gennari et al., 1994b). Soil pH can also influence acifluorfen sorption. At soil pH above p$K_a$ 3.5 (Roy et al., 1983), acifluorfen is predominantly negatively charged and would be expected to have little sorption in many soils. In soils with significant pH-dependent charge, increased sorption may result from increased anion exchange capacity. Lower water solubility of acifluorfen (solubility of acifluorfen acid = 120 mg L$^{-1}$, 25 °C) at reduced pH may also be a factor influencing retention. Protonation of the carboxyl group under acidic conditions could enhance hydrogen bonding (Pusino et al., 1993).

Acifluorfen sorption to two humic acids was inversely related to pH, but the magnitude of the pH effect varied between humic acids (Ruggiero et al., 1992). The work by Negre et al. (1995) studying effects of ethylene oxide sterilization on acifluorfen sorption in humic acid and whole soil substantiated these effects of pH and organic carbon on acifluorfen sorption. Gennari et al. (1994b) also observed a trend in increased acifluorfen sorption with decreasing pH when studying several soils of various physical and chemical properties.

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Acifluorfen can form ligand exchange bonds with metal ions such as Cu(II) (Kozlowski, 1990), but the extent to which this may occur in soil is not well-known. Pusino et al. (1991) found that, when acifluorfen was equilibrated with homoionic montmorillonitic clays, divalent and trivalent cations were extracted from the clay interlayer and deposited as precipitates on the clay surface. Acifluorfen sorption in soil was positively correlated with cation exchange capacity (CEC) (Pusino et al., 1993). Saturating soil with $Na^+$ reduced acifluorfen sorption, while $Ca^{2+}$ saturation increased sorption. This behavior may be due to the different solubilities of acifluorfen metal complexes: sodium acifluorfen is highly soluble, whereas calcium acifluorfen is less soluble ($K_{sp} \approx 1.5 \times 10^{-9}$). The likely binding site on the acifluorfen molecule for metal complexation is the carboxylate group (Kozlowski, 1990; Pusino et al., 1991), a contention supported by QSAR analysis of acifluorfen indicating that 70% of the negative molecular electrostatic potential is located in the vicinity of the $NO_2$-COOH region of the molecule (Nandihalli et al., 1992).

Existing research on acifluorfen sorption has ignored the time-dependent aspects of these reactions. Nevertheless, sorption kinetics may influence the fate and transport of chemical species in the soil environment and are important to assessing potential offsite transport and environmental impact. The primary objective of the present study was to characterize factors which influence sorption and sorption kinetics of acifluorfen in a wide variety of soils.

## MATERIALS AND METHODS

**Soils.** Soils used in these studies included Dundee loam and silty clay loam (fine-silty, mixed, thermic Aeric Ochraqualf), Dundee silt loam from long-term no-tillage (NT) and conventional tillage (CT) soybeans, Weswood silt loam (fine-silty, mixed, thermic Fluventic Ustochrept), Mahan loamy fine sand and fine sandy loam (clayey, kaolinitic, thermic, Typic Hapludult), Miami silt loam (fine-silty, mixed, mesic, Typic Endoaquoll) from NT or CT soybeans, Ships clay (very-fine, mixed thermic Udic Chromustert), Sharkey clay (very-fine, montmorillonitic, nonacid, thermic Vertic Haplaquept), and Lafitte organic muck (euic, thermic Typic Medisaprist). Organic

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**Table 1. Characteristics of the Soils**

| soil | pH, 1:1, $CaCl_2$ | clay content (%) | CEC (cmol(+)$kg^{-1}$) | organic carbon (g $kg^{-1}$) | sorption $K_d$[a] (L $kg^{-1}$) | sorption $K_{oc}$[b] (L $kg^{-1}$) | Freundlich $K_f$[c] | Freundlich $1/N$[c] |
|---|---|---|---|---|---|---|---|---|
| Dundee loam (0−10 cm) | 5.59 | 13.1 | 12.1 | 7.47 | 0.48 | 64.3 | 0.84(0.088) | 0.85(0.028) |
| Dundee silty clay loam (0−10 cm) | 5.08 | 35.2 | 19.5 | 7.48 | 0.56 | 74.9 | 0.89(0.062) | 0.87(0.019) |
| Dundee silt loam (CT) (0−5 cm) | 5.29 | 22.0 | 14.3 | 11.9 | 1.30 | 109.5 | 2.04(0.103) | 0.82(0.015) |
| Dundee silt loam (NT) (0−5 cm) | 5.13 | 22.0 | 16.7 | 22.4 | 3.15 | 140.5 | 5.22(0.252) | 0.80(0.016) |
| Lafitte muck (0−10 cm) | 4.10 | 20.0 | 78.0[d] | 191.3 | 89.6 | 468.4 | 92.1(3.09) | 0.90(0.035) |
| Mahan loamy fine sand (0−13 cm) | 4.20 | 4.7 | 3.8 | 12.0 | 1.66 | 138.0 | 2.64(0.129) | 0.80(0.015) |
| Mahan fine sandy loam (26−36) | 4.40 | 14.8 | 4.1 | 0.10 | 0.86 | 8600 | 1.22(0.098) | 0.86(0.023) |
| Miami silt loam CT (0−10 cm) | 6.16 | 40.0 | 14.1 | 19.0 | 1.10 | 57.9 | 1.64(0.328) | 0.80(0.055) |
| Miami silt loam NT (0−10 cm) | 6.36 | 40.0 | 15.6 | 35.0 | 1.80 | 51.4 | 2.34(0.144) | 0.87(0.018) |
| Sharkey clay (0−10 cm) | 6.00 | 61.0 | 43.7 | 16.9 | 2.16 | 128.2 | 3.24(0.107) | 0.82(0.010) |
| Ships clay (0−10 cm) | 7.50 | 50.0 | 40.1 | 8.34 | 0.61 | 73.1 | 0.98(0.156) | 0.82(0.043) |
| Weswood silt loam (0−10 cm) | 7.70 | 21.0 | 16.4 | 3.11 | 0.30 | 95.2 | 0.47(0.064) | 0.84(0.036) |

[a] Linearized sorption $K_d = x/m/C$, where $x/m$ = μmol $kg^{-1}$, $C$ = μmol $L^{-1}$. [b] $K_{oc} = K_d$/(kg of organic C/kg of soil). [c] $K_f = [x/m]/C^{(1/N)}$; numbers in parentheses are the asymptotic standard errors. [d] Value was obtained from published values of representative Lafitte soils (Clark and White, 1978).

carbon, pH, CEC (Rhoades, 1982; Hendershot and Duquette, 1986), and clay content of soils are presented in Table 1. Soils were air-dried and sieved to <2 mm size particles.

**Chemicals.** Technical grade acifluorfen (98% purity) and aminoacifluorfen (98.9%) were purchased from Chem Service (West Chester, PA). $CF^3$-Ring-UL-$^{14}C$-labeled acifluorfen (99%, specific activity 667 MBq $mmol^{-1}$) was provided by BASF Corp. (Research Triangle Park, NC). Both [$^{14}C$]acifluorfen and technical-grade acifluorfen were used without further purification. Herbicide stock solutions were prepared in methanol and stored at 4 °C in the dark until use. Appropriate concentrations of $^{14}C$ and technical-grade working solutions were prepared in 0.01 M $CaCl_2$ or deionized $H_2O$.

**Analysis.** Measurement of radioactivity in all sample solutions was by scintillation counting (Packard Tri-Carb 4000, Packard Instrument Co., Downers Grove, IL) after mixing with Ecolume scintillation cocktail (ICN, Costa Mesa, CA).

Selected samples were analyzed by high-pressure liquid chromatography (HPLC) with a Waters (Waters, Milford, MA) HPLC system (Model 510 pump, 712 WISP autosampler, 490E UV detector, System Interface Module) equipped with an Alltima C-18 reversed-phase column (5 μm, 250 mm) (Alltech, Deerfield, IL). Mobile-phase conditions were isocratic acetonitrile:$H_20$ (pH 3.2, $H_3PO_4$) (60:40) at 1 mL $min^{-1}$ flow rate. Acifluorfen and metabolites were monitored using UV detection (230 and 296 nm), and $^{14}C$-labeled analytes were monitored using a Beta-Ram detector (INUS Systems, Inc., Tampa, FL). Acifluorfen and aminoacifluorfen HPLC retention times (RTs) were 12.7 and 13.8 min, respectively.

In thin-layer chromatography (TLC) analysis, 50-μL sample aliquots were spotted on the pre-adsorbent area of silica gel TLC plates (20 × 20 cm, 250-μm silica gel, Whatman, Clifton, NJ). Plates were developed to 10 cm in a toluene:ethyl acetate:acetic acid:$H_2O$ (50:50:1:0.5, v/v/v/v) solvent system. Chromatograms were analyzed with a Bioscan System 200 Imaging Scanner (Bioscan, Washington, DC). Acifluorfen and aminoacifluorfen corresponded to $R_f$ values of 0.14 and 0.26, respectively.

**Sorption Equilibrium and Kinetics.** Batch techniques were used to characterize acifluorfen sorption. A soil:solution ratio of 1:4 was used with Lafitte due to high organic carbon content (Table 1), whereas a ratio of 1:2 was used with all other soils. Five grams of air-dry soil (2 g for Lafitte) were weighed into 25-mL Pyrex centrifuge tubes, 10 mL (8 mL for Lafitte) of acifluorfen solution was added, the tubes were sealed with Teflon-lined caps, and suspensions were equilibrated on a rotary shaker at 25 °C for prescribed periods of time. At the end of each shaking interval, samples were centrifuged 10 min (15*g*, 12 °C, Beckman J2-21, Beckman Instruments, Palo Alto, CA) and aliquots were counted for radioactivity. The difference between input solution concentation and supernatant concentration after shaking was attributed to sorption. Concurrent blanks indicated no herbicide sorption to the tubes and caps.

In the kinetics experiment, soils were treated with 5.86 μM $^{14}C$-labeled (19.6 Bq $mL^{-1}$) acifluorfen. Reaction times on the shaker were 15 min or 1, 3, 6, 12, 24, 48, 72, or 96 h. In the equilibrium experiment, soils were treated with varying concentrations of $^{14}C$-labeled acifluorfen (ranging from 2.6 to 65.8 μM, 19.5 Bq $mL^{-1}$) to establish isotherms. Approximate time to reach pseudo-equilibrium, as determined from the kinetics experiment, varied from 24 to 48 h depending on the soil; but for consistency, all soils in this experiment were equilibrated for 24 h. In both experiments, each treatment combination was replicated at least four times.

**Microbial Population Dynamics and Biodegradation during Equilibration.** The potential for microbial degradation of acifluorfen during equilibration was examined using Dundee silt loam (NT and CT) and Sharkey clay. Conditions of the kinetics experiment were duplicated, and serial dilutions of the resulting supernatants were spiral-plated on agar plates (Spiral Instruments, Bethesda, MD) to determine total and gram-negative bacteria in the supernatants (Reddy et al., 1995). Four replications were used.

A kinetics experiment was conducted at either 4 or 25 °C with Dundee silt loam (NT and CT) and Sharkey soils to ascertain the extent of apparent sorption possibly attributable to degradation. Other conditions were the same as in the previous 25 °C kinetics experiment. One solution with a concentration of 5.86 μM acifluorfen (39.3 Bq $mL^{-1}$) was used. Equilibration time periods were 1, 24, 48, 72, or 96 h, with four replications per soil and time period combination. At the end of each shaking interval, samples were centrifuged and aliquots were counted for radioactivity.

A more detailed study was conducted at 25 °C with Dundee silt loam CT soil to evaluate acifluorfen metabolism during incubation (incubation increments were 0, 24, 48, 96, and 144 h). Five grams (oven-dry equivalent weight) of field moist (16% w/w) soil were added to sterile 25-mL tubes, and the soil was treated with 8 mL of 30 μM acifluorfen (406 kBq $L^{-1}$) in 0.125 M, pH 7 potassium phospate buffer. Sufficient potassium phosphate buffer was added to bring the final volume to 10 mL. The samples were shaken for the previously specified intervals, and the aqueous phase was separated by centrifugation for analysis of acifluorfen and metabolites. The soils were then extracted with 10 mL of methanol followed by an extraction with 10 mL of methanol:0.5 N NaOH (95:5, v/v). Aliquots from each fraction were mixed with Ecolume scintillation cocktail and counted for total radioactivity. Extracts from each fraction were acidified with 1 N HCl, diluted with deionized water, filtered through a solid-phase C-18 extraction (SPE) column, and eluted with methanol. Acifluorfen and metabolites were analyzed using HPLC and TLC.

**Effect of $CaCl_2$ as a Background Solution.** Dundee silt loam CT and Sharkey clay soils were used to assess the effect of 0.01 M $CaCl_2$ background on acifluorfen sorption (Pusino et al., 1991, 1993). Soils were treated as described previously with 5.86 μM $^{14}C$-labeled (0.959 kBq $L^{-1}$) acifluorfen dissolved in either 0.01 M $CaCl_2$ or deionized $H_2O$. Samples were equilibrated for 24 or 96 h at 25 °C, centrifuged, and sampled as described previously. The supernatant was analyzed with HPLC.

After the supernatant was removed, soils were extracted twice with methanol:Tris buffer (0.05 M, pH 7) (80:20). The methanol extracting solution was buffered at pH 7 to minimize sorption due to protonation at low pH and to reduce extraction of humic material at basic pH. Solutions from the first methanol extraction were analyzed with HPLC with no further processing. Remaining methanol extracts were evaporated, diluted to 50 mL with acidified water (adjusted to pH 3 with $H_3PO_4$), filtered through C-18 SPE columns, and eluted with methanol. The eluted methanol samples were analyzed with TLC.

After the second extraction with methanol, soils were air-dried and ground with a mortar and pestle. Soil samples (0.3 g) were then combusted (Oxidizer 306, Packard Instrument Co.) to determine methanol-nonextractable $^{14}C$.

**Sorption of Aminoacifluorfen.** Sorption of aminoacifluorfen was evaluated in the Dundee CT and Sharkey soils. Four grams of air-dry soil were treated with 8-mL aminoacifluorfen solutions at concentrations ranging from 3.02 to 30.1 $\mu$M in deionized $H_2O$. Samples were equilibrated on a shaker for 24 or 96 h at 25 °C and centrifuged for 10 min at 15$g$ (12 °C), and supernatants were analyzed with HPLC methods described previously. Blanks containing no soil were run concurrently and indicated that sorption to Pyrex tubes and caps was minimal.

**Statistical Analyses.** A split plot design was used in all experiments. Analysis of variance statistical procedures were used to evaluate effects of variables such as soil and temperature. Standard error (s.e.) was used to indicate variability, and Fisher's least significant difference (LSD) test was used to separate means. Nonlinear regression was used to estimate model parameter coefficients.

**Kinetic Models.** The time-dependent increase in sorbed acifluorfen up to 48 h was described by a two-site equilibrium/kinetic model

$$S_1 = k_e C^{1/N} \quad (1)$$

$$dS_2/dt = k_f C^{1/N} - k_r S_2 \quad (2)$$

where $S_i$ is sorbed concentration ($\mu$mol kg$^{-1}$), $C$ is solution concentration ($\mu$M), $k_e$ (L kg$^{-1}$) is the coefficient for sorption at sites 1 exhibiting instantaneous equilibrium, $k_f$ (L kg$^{-1}$ h$^{-1}$) and $k_r$ (h$^{-1}$) are rate constants for sorption and desorption, respectively, from sites 2, $1/N$ accounts for sorption nonlinearity, and $t$ (h) is time. Further sorption of $^{14}C$ beyond 48 h may reflect irreversible sorption of acifluorfen or degradation and sorption of metabolites as well as parent compound. Extension of the two-site model to include a third type of site may account for the former. Alternatively, acifluorfen degradation and time-dependent sorption of metabolites may be qualitatively described by assuming first-order kinetics:

$$dP/dt = k_d C - k_p P \quad (3)$$

$$dS_P/dt = (v/m)k_p P \quad (4)$$

where $P$ is the solution concentration of all metabolic products ($\mu$M), $S_P$ is concentration of sorbed metabolite ($\mu$mol kg$^{-1}$), $k_d$ (h$^{-1}$) and $k_p$ (h$^{-1}$) are first-order rate constants for substrate degradation and metabolite sorption, respectively, $v$ is solution volume (L), and $m$ is sorbent mass (kg).

This two-site model (including extensions) was expressed in finite difference form and fitted to the data using nonlinear regression. To reduce the number of fitted parameters, the reaction order $1/N$ was taken from the sorption isotherm data (24-h reaction time). However, $k_e$, $k_f$, and $k_r$ were not constrained such that $k_e + k_f/k_r = K_f$ (Freundlich coefficient at 24-h reaction time) because $K_f$ may underestimate the equilibrium value.

## RESULTS AND DISCUSSION

**Factors Influencing Sorption.** Acifluorfen sorption was initially rapid, with from 49% (Weswood) to 93% (Lafitte) of total $^{14}C$ sorption which occurred taking place within 1 h. In all soils, acifluorfen sorption up to 48-h reaction time was adequately described by the two-site model (eqs 1 and 2). Sorption kinetics up to 48 h for most of the soils are illustrated in Figures 1–3, and model parameters obtained by fitting the data are given in Table 2.

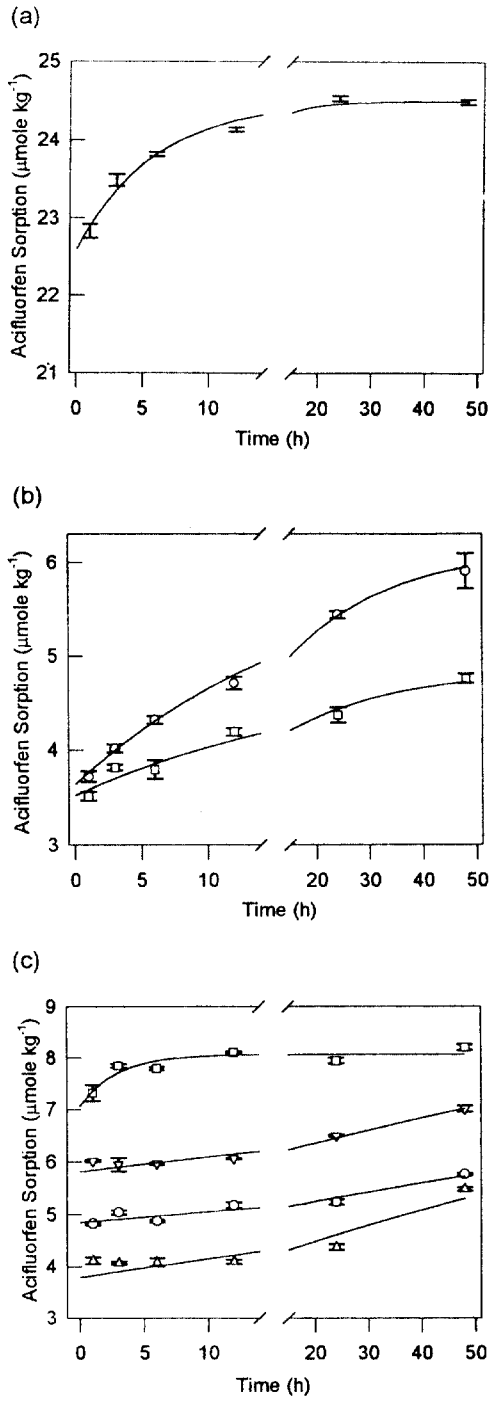


**Figure 1.** Acifluorfen sorption kinetics for (a) Lafitte muck soil; (b) Mahan soil sampled from the 0–13 cm (○) and 26–36 cm (□) depths; and (c) Dundee (CT, ○; NT, □) and Miami (CT, △; NT, ▽) soils. Bars associated with each symbol represent the standard error of each mean value. Curves represent best fits of data using eqs 1 and 2.

In light of apparent equilibrium conditions at 24–48 h and to minimize the potentially confounding effects of degradation, sorption capacity (Table 1; e.g., Figure 4) was evaluated after a 24-h shaking for all soils. The Freundlich sorption parameter ($K_f$) values ranged from

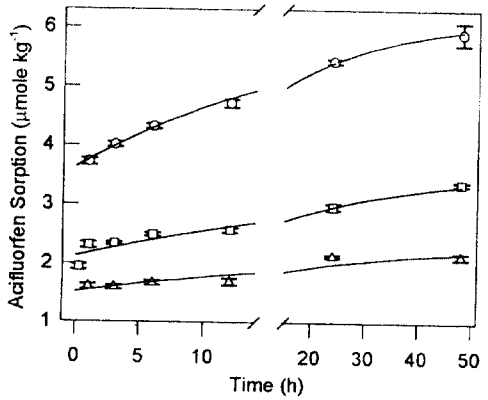


**Figure 2.** Effect of soil pH on acifluorfen sorption kinetics in three surface soils: Weswood silt loam (△), Mahan loamy fine sand (○), and Dundee silty clay loam (□). Bars associated with each symbol represent the standard error of each mean value. Curves represent best fits of data using eqs 1 and 2.

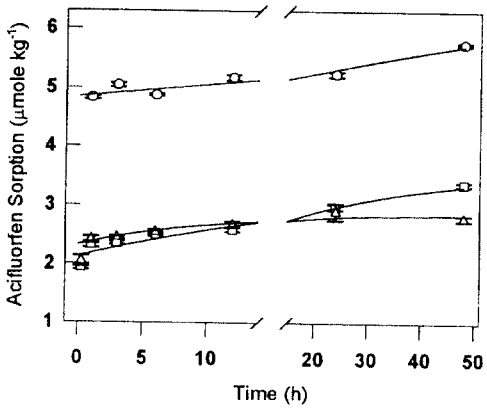


**Figure 3.** Comparison of acifluorfen sorption kinetics in Dundee loam (△), Dundee silt loam CT (○), and Dundee silty clay loam (□). Bars associated with each symbol represent the standard error of each mean value. Curves represent best fits of dat using eqs 1 and 2.

92.1 L $kg^{-1}$ (Lafitte) to 0.47 L $kg^{-1}$ (Weswood). All sorption isotherms were nonlinear and exponent ($1/N$) values less than 1 (Table 1), indicating decreasing fraction sorbed with increasing initial acifluorfen concentration. Variable acifluorfen sorption was attributed to several factors. Linear correlations indicated that organic carbon content ($r = 0.99$***), soil $H^+$ concentration ($r = 0.71$**), and CEC ($r = 0.78$***) were positively related to acifluorfen sorption (linearized $K_d$, Table 1). Multiple regression described these relationships as:

$$K_d = -1.05 + (0.102(\text{organic C}))^{**} + (12769(\text{CEC})[\text{H}])^{**}, \quad R^2 = 0.99^{**} \quad (5)$$

Sorptive capacity for the Lafitte muck was more than 1 order of magnitude higher than any other soil (Figure 1a and Table 1). Figure 1b compares sorption between two soil depths of Mahan having similar coarse texture and pH (Table 1) but greater organic carbon and acifluorfen sorption capacity in the surface soil. Higher acifluorfen sorption in the Dundee NT and Miami NT soils compared to their respective CT soils was also attributed to higher organic carbon (Figure 1c and Table 1). Higher organic carbon was also the probable reason for higher acifluorfen sorption in the Dundee CT and NT soils as compared to the other two Dundee soils (Table 1).

Soil pH can also influence acifluorfen sorption (Roy et al., 1983; Pusino et al., 1993; Ruggiero et al., 1992). At pH levels above its $pK_a$, acifluorfen is substantially dissociated and subject to negative repulsion in most soils. Protonation of the carboxyl group under acid conditions generates the free acid, thus enhancing hydrogen bonding of acifluorfen to soil components. We compared three surface soils with relatively low organic carbon contents but varying pH: Weswood silt loam is derived from alluvial calcareous material, Dundee silty clay loam is moderately acidic, and Mahan loamy fine sand is acidic (Table 1). Acifluorfen sorption in these soils was inversely related to soil pH (Figure 2, Table 1). Also, some acid soils exhibit higher anion exchange capacity, which could increase sorption of anionic moieties of acifluorfen. This may have been a factor contributing to acifluorfen sorption in the acidic Mahan surface and subsoil, where Fe oxides are abundant and the dominant clay component is kaolinitic.

Cation exchange capacity has been cited as a factor in acifluorfen sorption (Pusino et al., 1993), and a significant positive relationship between CEC and acifluorfen sorption was observed in the present study (eq 5). Because the effect of CEC was interactive with pH and organic carbon, an increase in CEC did not always result in a direct increase in acifluorfen sorption (Table 1). Pusino et al. (1993) found that acifluorfen sorption correlated with organic matter content but not with clay content. Although the source of negative charge in soil is from both organic matter and clay, the negative charge from organic matter may therefore be more important to acifluorfen sorption than the charge derived from clay. Comparisons of acifluorfen sorption among Dundee loam, Dundee silt loam CT, and Dundee silty clay loam soils were used to illustrate this point (Table 1). The Dundee soil developed from Mississippi alluvial material and possesses montmorillonitic clay. Cation exchange capacity increased with increasing clay content for the three soils, but acifluorfen sorption did not increase in the same order (Table 1, Figure 3). Acifluorfen sorption was similar for the soils with the lowest and highest clay content (Dundee loam and Dundee silty clay loam, respectively) but the same organic carbon content. The highest acifluorfen sorption occurred in the soil with the highest organic carbon content but medium clay content (Dundee silt loam CT) (Table 1, Figure 3).

Since Ca–acifluorfen complexes may form in soil (Pusino et al., 1993), we examined the effect of 0.01 M $CaCl_2$ background on acifluorfen sorption. Acifluorfen sorption was greater in solutions with 0.01 M $CaCl_2$ than in $H_2O$ for both soils (Table 3). Similarly, methanol-nonextractable $^{14}C$ increased with time for both $CaCl_2$ and $H_2O$ solutions, but tended to be greater for the $CaCl_2$ solutions (Table 3).

Sorption experiments commonly use a salt solution like 0.01 M $CaCl_2$ to mask small differences in ion contents among soils. An important effect of using 0.01 M $CaCl_2$ as an equilibrating solution is increased displacement of acidic cations and increased acidity (Peech, 1965). Koskinen and Cheng (1983) found that increasing salt ($CaCl_2$) concentrations of equilibrating solutions increased sorption of weak acidic herbicides because of lower pH. We believe that potential explanations for enhanced acifluorfen sorption in the $CaCl_2$ solutions (Table 3) were a combination of an increase in the protonated moiety of acifluorfen at lower pH and reaction of the anionic moiety of acifluorfen with exchangeable Al or Fe. We discounted Ca–acifluorfen precipitation as a major mechanism of increased sorp-

**Table 2. Parameters for the Two-Site Equilibrium/Kinetic Model (Eqs 1 and 2) Used To Describe $^{14}C$ Sorption up to 48 h in Several Soils**

| soil | $k_e$ (L/kg) | $k_f$ (L/(kg h)) | $k_r$ (1/h) |
|---|---|---|---|
| Dundee loam (0−10 cm) | 0.63 ± 0.02 | 0.01 ± 0.01 | 0.10 ± 0.04 |
| Dundee silty clay loam (0−10 cm) | 0.54 ± 0.06 | 0.02 ± 0.01 | 0.06 ± 0.06 |
| Dundee CT silt loam, 4 °C (0−5 cm)[a] | 1.84 ± 0.06 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| Dundee (CT) silt loam, 25 °C (0−5 cm) | 1.75 ± 0.05 | 0.01 ± 0.01 | 0.01 ± 0.02 |
| Dundee (NT) silt loam, 4 °C (0−5 cm)[a] | 4.40 ± 0.06 | 0.06 ± 0.01 | 0.03 ± 0.01 |
| Dundee (NT) silt loam, 25 °C (0−5 cm) | 3.52 ± 0.38 | 0.38 ± 0.28 | 0.29 ± 0.17 |
| Lafitte muck (0−10 cm) | 33.5 ± 2.0 | 3.96 ± 0.65 | 0.06 ± 0.01 |
| Mahan loamy fine sand (0−13 cm) | 1.18 ± 0.02 | 0.06 ± 0.01 | 0.04 ± 0.01 |
| Mahan fine sandy loam (26−36 cm) | 1.05 ± 0.04 | 0.03 ± 0.01 | 0.04 ± 0.02 |
| Miami (CT) silt loam (0−10 cm) | 1.26 ± 0.13 | 0.02 ± 0.02 | 0.01 ± 0.03 |
| Miami (NT) silt loam (0−10 cm) | 2.24 ± 0.06 | 0.02 ± 0.01 | 0.01 ± 0.01 |
| Sharkey clay, 4 °C (0−10 cm)[a] | 1.97 ± 0.12 | 0.02 ± 0.01 | 0.01 ± 0.01 |
| Sharkey clay, 25 °C (0−10 cm) | 1.85 ± 0.05 | 0.10 ± 0.02 | 0.09 ± 0.02 |
| Ships clay (0−10 cm) | 0.68 ± 0.02 | 0.01 ± 0.01 | 0.04 ± 0.02 |
| Weswood silt loam (0−10 cm) | 0.39 ± 0.03 | 0.01 ± 0.01 | 0.04 ± 0.04 |

[a] Data to 96-h reaction time was fitted.

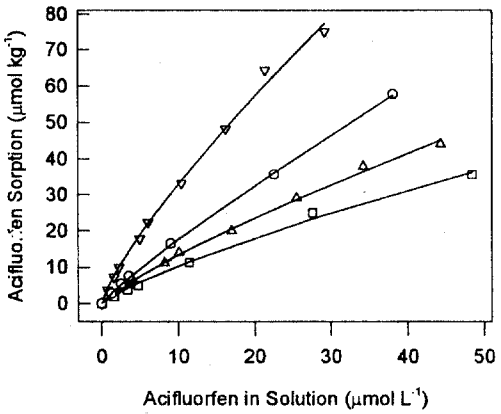


**Figure 4.** Freundlich sorption isotherms for acifluorfen showing the effects of tillage in Dundee silt loam (CT, △; NT, ▽) and Miami silt loam (CT, □; NT, ○) soils.

tion in our study for the following reasons. First, there was no evidence of precipitate formation observed in solutions with 0.01 M $CaCl_2$. Both water and $CaCl_2$ solutions were prepared from the same stock solution of [$^{14}C$]acifluorfen, and both final working solutions measured the same radioactivity. Had there been any significant precipitation, the radioactivity for the $CaCl_2$ solution would have been lower. The solutions in both $H_2O$ and $CaCl_2$ were stable under refrigerated conditions and, after several weeks of storage, measured the same radioactivity as initial readings. Also, according to Pusino et al. (1993), the $K_{sp}$ for Ca−acifluorfen is approximately $1.5 \times 10^{-9}$. For our conditions, it would have required an acifluorfen concentration of 387 $\mu$M to form Ca−acifluorfen precipitates, and our highest concentration (65.8 $\mu$M) was well below that point.

**Sorption Kinetics.** In several soils, sorption achieved a pseudo-plateau from about 24 to 48 h and then later increased. Representative data for acifluorfen sorption at 25 °C in Dundee CT, Dundee NT, and Sharkey soils are shown in Figure 5. Simple extensions of the two-site model to include nonequilibrium sorption at a third type of site did not adequately describe the continued increase in $^{14}C$ sorption. Since acifluorfen is subject to biotransformation, these $^{14}C$ sorption data probably include radiolabeled metabolites as well as the parent compound. Data for Dundee CT, Dundee NT, Miami CT, Miami NT, Sharkey, Ships, and Weswood soils were well described assuming acifluorfen degradation and metabolite sorption followed eqs 3 and 4.

**Biodegradation.** Little is published concerning acifluorfen degradation in soil. Gennari et al. (1994a) observed that, under anaerobic conditions, mixed enrichment cultures from soils with a history of acifluorfen exposure reduced acifluorfen to aminoacifluorfen when acifluorfen was the sole source of carbon. Inclusion of additional carbon sources such as sodium acetate promoted further degradation to other products. In our study, bacterial populations in soil suspensions of Sharkey and Dundee (NT and CT) soils indicated increased bacterial numbers, especially gram-negative, with time (Table 4). These bacteria may have transformed acifluorfen to a metabolite having a greater affinity for sorption to soil than acifluorfen. Although samples were well shaken, the rapid proliferation of bacteria may also have induced microaerophilic conditions which are potentially conducive to aromatic nitroreduction of acifluorfen.

The carboxyl and amino functional groups and positions on the amino-substituted ring of aminoacifluorfen are identical with the functional groups of anthranilic acid (2-aminobenzoic acid). Gaston et al. (1996) have shown that anthranilic acid has a high affinity for a Dundee and Sharkey soil. If the carboxyl and amino groups are the active sorption sites for the anthranilic acid, they may also contribute to sorption of a metabolite such as aminoacifluorfen. Another diphenyl ether herbicide, bifenox [methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate], was rapidly hydrolyzed in soil to the acid form (carboxylic ester hydrolysis), and the nitro group was reduced to amino (Ohyama and Kuwatsuka, 1983). They hypothesized that, for the bifenox metabolites, the sorption associated with the carboxyl and hydroxl groups was related to hydrogen bonding and van der Waals forces, while hydrophobic bonding contributed to sorption at the amino group. Similar enhanced affinity for soil of amino derivatives of several diphenyl ether herbicides was observed by Niki and Kuwatsuka (1976). In our study, aminoacifluorfen sorption isotherms for Dundee CT and Sharkey soils indicated a high sorption affinity (Dundee CT: $K_f = 47.2$, $1/N = 0.41$; Sharkey: $K_f = 41.3$, $1/N = 0.72$). Average total recoveries of aminoacifluorfen (combined sorption solutions and methanol extracts) were only 9.9% (s.e. 1.23) and 17.8% (s.e. 0.89) for Dundee CT and Sharkey soils, respectively. Further metabolism of aminoacifluorfen during the 24-h equilibration was indicated by the presence of an unidentified peak in methanol extracts, especially in the Sharkey soil. Area of the unidentified peak increased with increasing concentration of added aminoacifluorfen, and the peak was not present in untreated soil extracts.

**Table 3. Effect of $CaCl_2$ on Acifluorfen Sorption, Methanol-Nonextractable $^{14}C$, and Solution pH after 24- or 96-h Equilibration with Dundee CT and Sharkey Soils**

| soil | shaking time (h) | acifluorfen sorption ($\mu$mol kg$^{-1}$)[a] | | methanol-nonextractable $^{14}C$ (% of applied)[a] | | supernatant pH[b] | |
|---|---|---|---|---|---|---|---|
| | | 0.01 M $CaCl_2$ | water | 0.01 M $CaCl_2$ | water | 0.01 M $CaCl_2$ | water |
| Dundee CT | 24 | 5.04a | 2.81b | 0.16a | 0.03a | 6.69 | 7.19 |
| | 96 | 7.99a | 5.73b | 20.8a | 13.2b | 6.60 | 7.24 |
| Sharkey | 24 | 5.65a | 1.99b | 7.91a | 6.08b | 6.63 | 7.53 |
| | 96 | 7.31a | 4.58b | 13.3a | 10.1b | 6.25 | 7.07 |
| average | | 6.50a | 3.78b | 10.5a | 7.35b | | |

[a] Within a row and for each parameter, means followed by the same letter are not significantly different. LSD $\alpha = 0.05$. [b] pH was measured in supernatants pooled over replications.

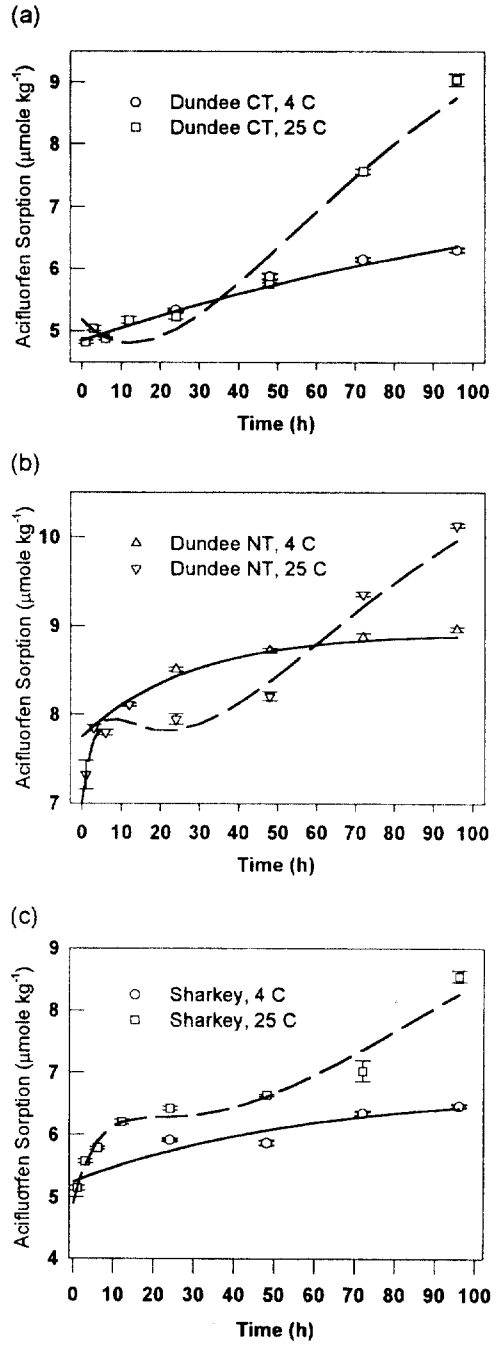


**Figure 5.** Effect of equilibrating temperature on kinetics of acifluorfen sorption in (a) Dundee CT, (b) Dundee NT, and (c) Sharkey soils. Bars associated with each symbol represent the standard error of each mean value. Curves depicted as dashed lines (25 °C) represent best fits of data using eqs 1–4. Curves depicted as solid lines (4 °C) represent best fits of data using eqs 1 and 2.

**Table 4. Changes in Bacterial Populations during 96-h Shaking**

| time (h) | total bacteria (log CFU mL$^{-1}$)[a] | gram-negative (log CFU mL$^{-1}$)[a] |
|---|---|---|
| | Sharkey | |
| 1 | 6.46b | <4.20d |
| 24 | 7.84a | 5.05c |
| 48 | 7.84a | 6.31b |
| 72 | 7.86a | 6.51ab |
| 96 | 7.89a | 6.59a |
| | Dundee CT | |
| 1 | 6.74c | 4.70d |
| 24 | 7.26b | 5.78c |
| 48 | 7.67a | 6.34b |
| 72 | 7.66a | 6.54ab |
| 96 | 7.61a | 6.73a |
| | Dundee NT | |
| 1 | 6.92c | 4.88c |
| 24 | 7.30b | 5.82b |
| 48 | 7.75a | 6.60a |
| 72 | 7.84a | 6.67a |
| 96 | 7.78a | 6.65a |

[a] Within a soil, means followed by the same letter are not significantly different. LSD $\alpha = 0.05$.

Since sorption was based upon $^{14}C$ remaining in solution after equilibration, other avenues of potential loss need to be addressed. One potential for loss of $^{14}C$ from the mass balance equation is in the form of $^{14}CO_2$ (mineralized acifluorfen). However, upon the basis of results from Gaston and Locke (1996), it is doubtful that sufficient degradation occurred during this period to result in complete metabolization to $^{14}CO_2$. Gennari et al. (1994a) did not observe ether cleavage of acifluorfen in mixed enrichment cultures incubated for 56 d.

To further evaluate potential microbial degradation during sorption, Sharkey and Dundee NT and CT soils were equilibrated with acifluorfen solution for various time intervals at either 4 or 25 °C. Although factors related to sorption thermodynamics may be involved, reduced acifluorfen sorption at the lower temperature is consistent with what might be expected with lower microbial degradation (Figure 5).

Analysis of methanol extracts (after sorption) with the TLC toluene system for Dundee CT and Sharkey soils showed a major $^{14}C$ peak, coeluting with acifluorfen ($R_f$ 0.14), and a minor peak ($R_f$ 0.26) that coeluted with aminoacifluorfen. The area represented by the minor peak increased with equilibration time (24–96 h), especially in the Dundee CT (Dundee CT, 0–14%; Sharkey, 0–3.9%). HPLC analysis of sorption supernatants and methanol extracts for both equilibration periods showed that most of the extractable $^{14}C$ coeluted with acifluorfen. There were other minor peaks of radioactivity in some 96-h methanol extracts (4–13% of radioactivity), most consistently at a RT of 13.8 min, which coincided with an aminoacifluorfen standard.

**Table 5. Degradation of Acifluorfen in Dundee CT Silt Loam Soil**

| | % of total $^{14}C$ Applied | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | aqueous extracts[a] | | | methanol extracts[a] | | | | |
| (time (h) | acifluorfen | aminoacifluorfen | total | acifluorfen | aminoacifluorfen | other[b] | total | nonextract[a] |
| 24 | 78.1(0.93) | <0.1(<0.001) | 78.2 | 15.1(0.40) | <0.1(<0.01) | 2.0(0.07) | 17.2 | 1.3(0.11) |
| 48 | 71.5(0.51) | <0.1(<0.001) | 71.5 | 16.8(0.25) | 0.7(0.04) | 2.2(0.09) | 19.7 | 4.0(0.14) |
| 96 | 49.1(0.46) | 4.2(0.39) | 53.3 | 14.5(0.40) | 10.4(0.32) | 2.4(0.08) | 27.2 | 9.8(0.23) |
| 144 | 22.1(1.17) | 12.7(0.24) | 34.7 | 8.4(0.73) | 19.2(1.14) | 2.6(0.24) | 29.7 | 17.1(0.57) |

[a] Numbers in parentheses are standard errors. [b] Decarboxy acifluorfen and 4-(trifluoromethyl)-2-chlorophenol.

The study evaluating acifluorfen degradation in Dundee CT soil corroborated much that was observed previously. Acifluorfen accounted for 97–98% of extractable (aqueous and methanolic) $^{14}C$ after 24- and 48-h shakings, respectively (Table 5). Minor peaks in extracts coeluted with aminoacifluorfen, decarboxyacifluorfen, and 4-trifluoromethyl-2-chlorophenol. Aminoacifluorfen was the primary metabolite observed in extracts from the 96- and 144-h shakings. Nonextractable $^{14}C$ ranged from 1.3 to 17.1% during the 24–144-h shakings (Table 5). No significant quantity of $^{14}C$ was extracted using 5:95 NaOH:methanol until the 144-h shaking, when 4.3% was extracted (data not shown).

## SUMMARY AND CONCLUSIONS

Several soils of diverse character were used to study factors which influence acifluorfen sorption. Sorption was rapid for all soils (percentage of total sorption ranged from 49% for Weswood to 93% for Lafitte within 1 h. Most soils reached a pseudo-equilibrium after 24–48 h of equilibration. Increases in apparent acifluorfen sorption at equilibration times longer than 24–48 h may reflect acifluorfen degradation and metabolite sorption. Further investigations comparing sorption at 4 and 25 °C indicated that microbial metabolism was occurring for some soils at equilibration times longer than 24–48 h. HPLC and TLC analyses of methanol extracts from soils after equilibration for 24 and 96 h showed that a metabolite, aminoacifluorfen, was present in the 96-h samples. Sorption studies with aminoacifluorfen indicated that it has a high affinity for soil, a factor which may help explain the irreversibility of sorption observed in these experiments. Capacity for acifluorfen sorption (24-h equilibration) in these soils in decreasing order was Lafitte ≫ Dundee NT > Sharkey > Miami NT > Mahan (0–13 cm) > Dundee CT > Miami CT > Mahan (26–26 cm) > Ships > Dundee scl = Dundee l > Weswood. Varying levels of acifluorfen sorption among soils were attributed to (a) organic carbon, (b) pH, and (c) CEC.

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# Ion-Pair Reverse Phase Liquid Chromatographic Determination of Sodium Acifluorfen in Feed

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**Ion-pair reverse phase liquid chromatography (LC) and UV detection at 280 nm have been used to determine sodium acifluorfen (sodium-5-[2-chloro-4-(trifluoromethyl)-phenoxy]-2-nitrobenzoate), an experimental diphenyl ether herbicide, in dog feed. Sodium-5-(2,4-dichlorophenoxy)-2-nitrobenzoate is used as the internal standard. The feed is homogenized in 0.01N HCl, followed by ethyl acetate extraction, and centrifugation. The organic layer is removed and evaporated and the residue is reconstituted in methanol and filtered before LC analysis (mobile phase methanol–water (58 + 42), 0.005M in tetrabutylammonium phosphate and 0.045M in $(NH_4)_2HPO_4$, at pH 7.4). The ion-pair technique offers a high degree of control over the retention characteristics of the herbicide and internal standard. The use of the internal standard permits precise and accurate quantitation and substantially reduces analysis time compared with the external standard method.**

Sodium acifluorfen (sodium-5-[2-chloro-4-(trifluoromethyl)-phenoxy]-2-nitrobenzoate) is a selective herbicide used for pre- and post-emergence residual weed control of a wide spectrum of annual broadleaf weeds and grasses in soybeans, peanuts, and other large seeded legumes. Sodium acifluorfen is the active ingredient in Blazer® (Rohm and Haas, United States and Brazil), and in Tackle® (formerly owned by Mobil Oil Corp., now owned by Rhône-Poulenc Chemical Co.). Tackle is currently sold in Brazil and an experimental use permit has been granted for its use in the United States. Toxicity testing is under way for the purpose of obtaining full registration in the United States. To assist in the safety assessment program for Tackle, we developed a reliable liquid chromatography (LC) assay procedure to provide assurance of herbicide homogeneity and requisite concentration in dog feed.

The use of LC for determining sodium acifluorfen in feed was investigated as an alternative to the residue method reported by Adler et al. (Adler, I. L., Augenstein, L. L., & Rogerson, T. D. (1978) *J. Assoc. Off. Anal. Chem.* **61,** 1456–1458), which specifies a gas chromatographic procedure. Early attempts used simple acid extraction procedures and conventional reverse phase LC techniques; however, the sodium acifluorfen peak could not be satisfactorily resolved from feed components in a reasonable time. Rather than incorporate more elaborate and time-consuming sample preparations to overcome this, an ion-pair LC technique was developed to effect the selectivity required for separation and analysis.

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## METHOD

### *Reagents*

(a) *Methanol and ethyl acetate.*—Baker Analyzed LC grade.

(b) *Water.*—Distilled and deionized.

(c) $(NH_4)_2HPO_4$.—Baker Analyzed.

(d) *Mobile phase.*—Methanol–water (58 + 42 v/v), 0.005M with respect to tetrabutylammonium phosphate (TBA) and 0.045M with respect to dibasic ammonium phosphate (pH of aqueous phase = 7.4). Filter aqueous solution and methanol through Whatman 0.45 μm discs and degas under reduced pressure before use.

(e) *Ion-pair reagent.*—Use as received. Tetrabutylammonium phosphate (Eastman Kodak Co., Rochester, NY 14650).

(f) *Standards.*—Both acifluorfen (99% purity) and internal standard (98% purity) were supplied by Mobil Chemical Co. as the free acids.

### *Apparatus*

*Liquid chromatograph.*—Hewlett-Packard 1084B equipped with variable wavelength detector operated at 280 nm (sample) and 430 nm (reference). Chromatographic columns: 150 × 4.6 mm id, 5 μm particle diameter Altex C-18 reverse phase or 250 × 4.6 mm id, 5 μm particle diameter C-8 reverse phase DuPont Zorbax operated at 1.5 mL/min flow rate. Maintain column oven at 30°C. Instrument is equipped with variable volume autoinjector; typical injection volumes are 10–50 μL depending on concentration of sodium acifluorfen in feed.

### *Preparation of Feed Samples*

Accurately weigh 1.00 g animal feed (Purina 5007 Dog Chow) in disposable plaster weighboat and transfer feed to 30 mL Corex centrifuge tube. Add 500 μL sodium-5-(2,4-dichlorophenoxy)-2-nitrobenzoate (internal standard) solution containing amount of material equal to nominal per gram concentration of sodium acifluorfen in feed. Add 10 mL 0.01 HCl to tube and homogenize on Polytron homogenizer for 30 s. Add 5–10 mL ethyl acetate to aqueous acid slurry and repeat homogenization for 30 s. Cover Corex tube with Parafilm and centrifuge at 3500 × *g*, for 10 min. Remove ethyl acetate layer with disposable Pasteur pipet and transfer to 13 × 100 mm disposable culture tube; evaporate ethyl acetate under gentle stream of nitrogen. Reconstitute residue in culture tube with 2 mL methanol and vortex-stir briefly. Filter reconstituted residue through disposable 0.2 μm disc (Gilman Acrodisc 4192). Transfer clear filtrate to appropriate septum-sealed LC vials for analysis.

### *Preparation of Standards*

(**a**) *Sodium acifluorfen and internal standard stock solutions.*—Prepare 100 mL aqueous standard solutions of sodium acifluorfen and sodium-5-(2,4-dichlorophenoxy)-2-nitrobenzoate at each dosage level from the free acids. Accurately weigh sufficient amounts of acids to give final concentrations of sodium salts of 900, 600, and 40 mg/100 mL. Dissolve the acids in 25–50 mL water containing an equimolar amount of sodium hydroxide. Adjust final pH of solution to 8.5 with 1.0N HCl and 1.0N NaOH before filling flask (100 mL) to mark.

(**b**) *Sample standards.*—Accurately weigh 1.00 g control feed and transfer to 30 mL Corex centrifuge tube. Add equal amounts (500 μL) of sodium acifluorfen and internal standard at appropriate dosage level. Prepare sample in manner described above.

### *Calculations*

Daily analyze sample standard data to determine correction factor reflecting deviation from the ideal, i.e., area sodium acifluorfen peak/area internal standard peak = 1.00, due to detector response, absorptivity, chemical selectivity, chemical purity, etc. Determine final concentration of sodium acifluorfen in feed using equation:

$$\text{SA, ppm} = (\text{area SA}/\text{area IS}) \times \text{CF} \times \text{dosage level, ppm}$$

where SA = sodium acifluorfen; IS = internal standard; CF = correction factor.

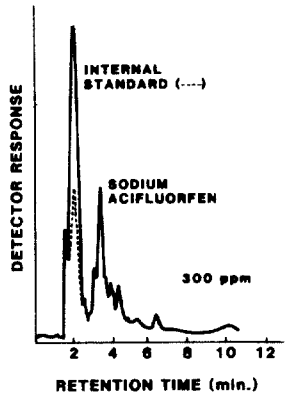


**Figure 1. Conventional reverse phase LC chromatograms (methanol–water (58 + 42); Altex C-18) of feed extract containing 300 ppm sodium acifluorfen and internal standard (---).**

## Results and Discussion

The sample preparation procedure described consists of acidification of the feed to generate the free acid (acifluorfen) followed by extraction into ethyl acetate. Of the several different methods evaluated, the acid–ethyl acetate procedure was somewhat superior in terms of recovery and reproducibility. Initially, a gradient reverse phase LC method was used in the assay; however, the time required per analysis (approximately 20 min) proved impractical as the daily number of required analyses increased. Isocratic reverse phase LC (methanol–water (58 + 42)) showed little promise of being able to resolve the acifluorfen and internal standard peaks from the numerous feed component peaks in an acceptable time (Figure 1, 300 ppm feed fortification). Addition of TBA and buffer [$(NH_4)_2HPO_4$] to the mobile phase, however, allows a high degree of control over the retention volume of the reversible ion-pair complex formed between TBA and the acifluorfen (and internal standard) anion. When the same sample (Figure 1) is analyzed using a methanol-water (58 + 42), 0.005M TBA and 0.045M $(NH_4)_2HPO_4$ solvent system, the retention time is 7.0 min for the TBA–acifluorfen complex and 5.7 min for the TBA–internal standard complex. Figure 2 shows the ion-pair reverse phase LC chromatogram of the sample (300 ppm) and 2 other feed samples fortified at 4500 and 20 ppm. The acifluorfen peak and internal standard are well resolved from one another and from the extractable feed component peaks observed near the solvent peak. The internal standard, sodium-5-(2,4-dichlorophenoxy)-2-nitrobenzoate, was chosen because of its structural similarities



Tabl

| Assay No. |
|---|
| 1 |
| 2 |
| 3 |
| 4 |
| Mean |
| SD |
| RSD, % |

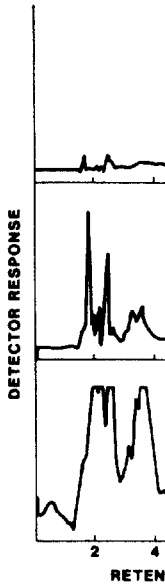


**Figure 2. Ion-pair reve (methanol–water (58 0.045M in $(NH_4)_2HPO_4$ extracts containing sod ternal standard (I.S.**

to sodium acifluorfe comparable pKa (3.5). in Figure 2 at a nomir (i.e., complete ionizat sufficient and practica the assay and the san eries of sodium acifluo were evaluated at the cussed above. The re age recoveries of 86% ( and 87% (20 ppm) wit tions of 0.7, 4.2, and 1

The assay has been u food samples from ch icity studies on sodiu

**Table 1. Recoveries of sodium acifluorfen standards from fortified dog food**

| | Spike level, ppm | | | | | |
|---|---|---|---|---|---|---|
| | 4500 | | 300 | | 20 | |
| Assay No. | Found, ppm | Rec., % | Found, ppm | Rec., % | Found, ppm | Rec., % |
| 1 | 3840 | 85 | 272 | 91 | 17.5 | 88 |
| 2 | 3870 | 86 | 258 | 86 | 17.2 | 86 |
| 3 | 3840 | 85 | 245 | 82 | 17.5 | 88 |
| 4 | 3870 | 86 | 258 | 86 | 17.4 | 87 |
| Mean | 86 | | 86 | | 87 | |
| SD | 0.58 | | 3.68 | | 0.96 | |
| RSD, % | 0.7 | | 4.2 | | 1.0 | |

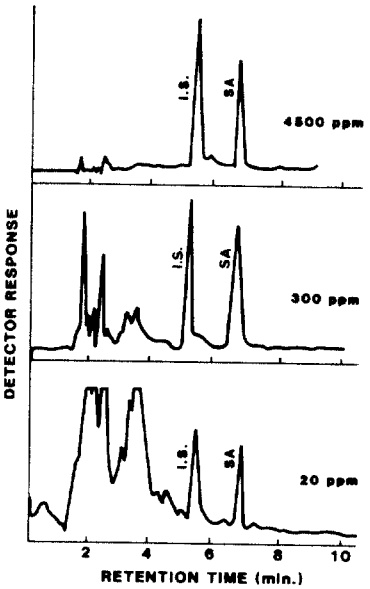


**Figure 2. Ion-pair reverse phase LC chromatograms (methanol–water (58 + 42), 0.005M in TBA and 0.045M in $(NH_4)_2HPO_4$; Altex C-18) of food sample extracts containing sodium acifluorfen (SA) and internal standard (I.S.) at 4500, 300, and 20 ppm.**

to sodium acifluorfen, and in particular, its comparable pKa (3.5). The separation observed in Figure 2 at a nominal mobile phase pH of 7.4 (i.e., complete ionization) was found to be both sufficient and practical in terms of the nature of the assay and the sample throughput. Recoveries of sodium acifluorfen using this procedure were evaluated at the 3 fortification levels discussed above. The results (Table 1) show average recoveries of 86% (4500 ppm), 86% (300 ppm), and 87% (20 ppm) with relative standard deviations of 0.7, 4.2, and 1.0%, respectively.

The assay has been used for the analysis of dog food samples from chronic and subchronic toxicity studies on sodium acifluorfen. Figure 2 shows representative chromatograms of dog food samples fortified at various levels from a subchronic study. A total of 156 samples and 26 blanks (0 ppm) were analyzed in duplicate. In addition, 23 sample standards were prepared at the 3 fortification levels (4500, 300, and 20 ppm) to generate the correction factor used in determining the sodium acifluorfen levels of the feed (see *Method* section). The correction factor represents the ratio of peak areas for sodium acifluorfen and internal standard fortified in the sample standards, and its standard deviation is a good measure of the overall precision of the assay. Table 2 includes the statistics for the correction factor from sample standards prepared at each dosage level during the study.

**Table 2. Statistical analysis of correction factors derived from sample standards**

| | Fortification level, ppm | | |
|---|---|---|---|
| Statistic | 20 | 300 | 4500 |
| No. sample standards | 8 | 8 | 7 |
| Data points (injections) | 26 | 50 | 22 |
| Mean correction factor[a] | 1.00 | 0.93 | 0.89 |
| SD | 0.05 | 0.05 | 0.05 |
| Rel. SD, % | 5.0 | 5.4 | 5.6 |

[a] Mean correction factor = area sodium acifluorfen/area internal standard peak for control feed samples fortified with equivalent amounts of the 2 compounds and prepared in a manner identical with actual samples.

## *Conclusion*

Ion-pair reverse phase LC offers a high degree of control over the retention characteristics of the herbicide, sodium acifluorfen, and the internal standard, sodium-5-(2,4-dichlorophenoxy)-2-nitrobenzoate. This selectivity minimizes sample preparation before analysis, and combined with the use of the internal standard allows for a high degree of precision and accuracy in the assay.

**APPENDIX 5: USE PROFILE FOR SODIUM ACIFLUORFEN**

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# APPENDIX 6: RESPONSE TO EPA REQUEST FOR INFORMATION ON POTENTIAL ALTERNATIVES TO SODIUM ACIFLUORFEN

## APPENDIX 6: RESPONSE TO EPA REQUEST FOR INFORMATION ON POTENTIAL ALTERNATIVES TO SODIUM ACIFLUORFEN

In its overview document, EPA has presented a list of potential alternatives to sodium acifluorfen in the soybean, peanut and rice markets. BASF believes that sodium acifluorfen products serve an essential role in these markets. Even though use, especially in the soybean market, has declined over the past several years due to the introduction of Roundup Ready soybeans, sodium acifluorfen products meet a need for weed problems not well-controlled by Roundup or other herbicides.

RICE

Acifluorfen controls a range of broadleaf weeds in rice that reduce yield by direct competition and, more importantly, reduce grade if they produce dark-colored seeds. Hemp sesbania, smartweed and morningglory species are black-seeded weeds and are all controlled by acifluorfen.

Acifluorfen is an important tool for the U.S. rice grower primarily because it provides superior control of hemp sesbania. In fact, acifluorfen, propanil and 2,4-D are the only herbicides labeled for mid-season control of this troublesome weed. Acifluorfen/propanil combinations are preferred over 2,4-D if cotton or other sensitive crops are nearby.

PEANUTS

Acifluorfen has the broadest spectrum among peanut herbicides for post-emergence broadleaf weed control. Blazer alone provides control of 10 of the top 15 broadleaf weeds. A commonly used treatment in peanuts is a pre-mix of acifluorfen and bentazon (Storm herbicide) which controls 13 of the top 15 broadleaf weeds in peanuts. Additionally, with the exception of an 18 month rotation to root crops, peanut growers have total rotational crop flexibility with acifluorfen.

SOYBEANS

Sodium acifluorfen (Ultra Blazer herbicide) is used most frequently in soybeans as a tank-mix partner or in a sequential program with other herbicide chemistries for control of morningglory species, ALS/AHAS-resistant Amaranthus (including common waterhemp), ragweed species, and kochia.

Common waterhemp is gradually becoming one of the most widespread and hard to control weed problems in Midwest soybeans (approximately 3 million acres were treated for waterhemp in 1996 versus 17 million acres in 2000). Total acres treated for Amaranthus species, in general, has increased from roughly 21 million acres in 1996 to 41 million acres in 2000. The widespread use of ALS-inhibiting herbicides and increased reliance on glyphosate for total weed control in Roundup Ready soybeans have

contributed to the Amaranthus weed shifts. Morningglory species, ragweed species, and other weed species shifts are likely to occur with continued reliance on glyphosate. The availability of effective alternative mode of action herbicides such as acifluorfen is critical to maintaining the long term effectiveness of weed management systems in soybeans.

Sodium acifluorfen is superior to imidazolinone herbicides (imazaquin, imazamox, imazethapyr) for control of morningglory species. Sulfonylurea herbicides (chlorimuron and cloransulam) control morningglory, but do not offer the same degree of rotational crop flexibility as acifluorfen (i.e., cloransulam has a 30 month rotational crop interval for sugar beet and sunflower; chlorimuron has a 30 month interval for sugar beet and potato).

ALS/AHAS – inhibiting herbicides are no longer the herbicides of choice for control of *Amaranthu*s and ragweed species due to the spread of herbicide resistant biotypes. A diphenyl ether herbicide such as acifluorfen provides excellent control of these troublesome weeds.